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PLASMA DISAPPEARANCE
OF UNLABELLED INSULIN
IN MAN

With special regard
to the role of the liver

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With special regard to the role of the liver

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The dissertation is based on the following papers:

- I. Tranberg, K.-G. & Dencker, H.: Modeling of plasma disappearance of unlabeled insulin in man. *Am. J. Physiol.* 235:E577-E585, 1978.
- II. Tranberg, K.-G.: Hepatic uptake of insulin in man. Submitted for publication, 1979.
- III. Tranberg, K.-G. & Thorell, J.I.: Extrahepatic splanchnic metabolism of insulin, glucose, and FFA. Submitted for publication, 1979.
- IV. Tranberg, K.-G. & Thorell, J.I.: Variation of plasma disappearance of unlabeled insulin: studies with portal and peripheral infusions. Submitted for publication, 1979.
- V. Tranberg, K.-G. & Hagander, P.: Rates of insulin release and disappearance in man: variation with the plasma insulin level. Submitted for publication, 1979.

In the text these papers are referred to by their Roman numerals.

GLOSSARY OF SYMBOLS

D	dose of insulin, $\mu\text{U/kg}$
I_a	concentration of arterial plasma insulin above the pretest baseline after infusion of insulin, $\mu\text{U/ml}$
I_{po}	concentration of portal plasma insulin above the pretest baseline after infusion of insulin, $\mu\text{U/ml}$
I_a^{ss}	concentration of arterial plasma insulin in the steady-state, $\mu\text{U/ml}$
I_{po}^{ss}	concentration of portal plasma insulin in the steady-state, $\mu\text{U/ml}$
APD	arterial-portal concentration difference, $\mu\text{U/ml}$
PCR	plasma clearance rate in the non steady-state, $\text{ml min}^{-1} \text{ kg}^{-1}$
PCR_{ss}	plasma clearance rate in the steady-state, $\text{ml min}^{-1} \text{ kg}^{-1}$
$\bar{t}$	mean transit time, min
ADV	apparent (total) distribution volume, ml/kg
FDR	fractional disappearance rate ($1/\bar{t}$), min^{-1}
k_{ij}	fractional transfer rate constant to compartment i from compartment j, min^{-1}
k_{el}	overall net fractional transfer rate constant from plasma to extravascular compartment(s), min^{-1}
APV	apparent plasma volume, ml/kg
FH	first pass fractional hepatic uptake of insulin
ESB	extrahepatic splanchnic bed (for insulin this notation comprises the extrahepatic and the extra-pancreatic bed)
PPF	portal plasma flow, $\text{ml min}^{-1} \text{ kg}^{-1}$
SER	simulated early insulin response, representing the net total rise of arterial plasma insulin after insulin administration, $\mu\text{U/ml}$
IVGTT	intravenous glucose tolerance test

- k_G blood glucose disappearance rate during the IVGTT,
%/min
- v superscript denoting the portal route of infusion,
or secretion, of insulin into the portal vein
- $\wedge$ superscript denoting the peripheral route of in-
fusion

INTRODUCTION

During the last two decades the process of insulin secretion has been extensively studied, and a large number of factors have been shown to affect the secretory rate (1-7). Studies on the metabolism of insulin have not only been relatively sparse, but also conflicting (8-27). As a consequence, the concept has emerged that variation of the clearance rate has a negligible influence on the plasma insulin level and that changes in the insulin level predominantly, or solely, reflect variation of the pancreatic secretion rate (28-33). But recently studies in vitro on the binding of insulin to plasma membrane receptor(s) have raised the possibility that insulin binding and degradation, and thus the plasma insulin level, is to some extent regulated by insulin itself as well as by other factors (34-44). In addition, studies in vitro frequently suggest that binding of insulin to receptors is correlated with the biological action of insulin though there are many situations in which this coupling is absent and/or where the binding and effect of insulin change in opposite directions (36, 37, 39, 45, 46). Since studies in vivo have not kept pace with those in vitro it is unknown to what extent data obtained in vitro may explain the behaviour of insulin in the intact organism. The physiologic significance of data obtained in vitro has been questioned (47-51).

The development of the present study illustrates our lack of knowledge of the behaviour of insulin in circulating blood. Thus, in addition to determining the hepatic uptake of insulin, the studies were primarily designed to measure the hepatic and peripheral effects of insulin and to relate these effects to the insulin secretion rate and the glucose tolerance within the same individual. But much of our data did not agree with the prevailing concepts of the clearance of insulin from plasma, and we therefore thought it important to analyse our data on insulin kinetics in detail.

The time-concentration relationship of insulin in plasma after intravenous administration has been studied and analysed in many ways. This has led to a considerable divergence of opinion concerning the manner and the rate of insulin disappearance (8-27). Radioiodinated insulins are frequently used but the validity of the results is debatable (9, 11, 14-16, 23). As for the uptake of insulin in the liver, most authors conclude that it is substantial, but the extent of this uptake appears highly variable (8, 9, 52-64). Nor is it clear whether changes in insulin and/or glucose, or other compounds, are accompanied by any change in the hepatic uptake of insulin (8, 9, 52-64). All insulin secreted into the blood enters the portal vein and passes through the liver at concentrations that may be considerably

higher than in the systemic circulation. The liver therefore has a good possibility to modify the distribution and removal of plasma insulin and to influence peripheral insulin levels. Variation in the magnitude of the hepatic uptake of insulin may be an important factor in the overall regulation of glucose and other nutrients (65-68). Except for one study with labelled insulin in anaesthetized patients (57) and one study in patients with severe liver disease (60), the hepatic insulin uptake has never before been determined in humans. The extrahepatic splanchnic bed is another metabolically active region receiving a large fraction of the cardiac output and also offering methodological difficulties. As for the exchange and effect of insulin across the extrahepatic splanchnic bed, previous studies, in animals, have given results that differ both qualitatively and quantitatively (55, 59, 69-73).

The objectives of the present investigation were to

- a) elucidate the kinetic properties of the disappearance of unlabelled insulin from plasma,
- b) determine the hepatic uptake of insulin at normoglycemia and moderate hyperglycemia,
- c) reveal whether there is an uptake of insulin across the extrahepatic splanchnic bed,
- d) find out whether the disappearance rate of plasma insulin is related to the effect of insulin on blood glucose and plasma FFA,
- e) find factors that may be important for variation in the disappearance of insulin, and
- f) establish to what extent the clearance of insulin from plasma is responsible for the plasma insulin level.

MATERIAL AND METHODS

Subjects

In all, 73 patients with, and 17 patients without, a trans-umbilical portal catheter were examined. Most patients were old, the mean age being 62. They had normal fasting blood glucose concentrations and standard liver and kidney laboratory tests gave normal values. Body weights ranged from 86 to 141 % of the ideal (Tables of Metropolitan Life Insurance Company). None had previous endocrine disease and none was receiving drugs known to disturb insulin or carbohydrate metabolism. The patients were in a good general condition at the time of the study. Most patients with a transumbilical portal catheter were to be, or had been, treated because of carcinoma of the colon. Patients without a portal catheter had a variety of surgical diagnoses, mostly inguinal hernia. None had demonstrable metastases in the liver or elsewhere, except for spread to regional lymph nodes. Portography (74) did not reveal portosystemic shunting.

The portal vein was entered extraperitoneally via the umbilical cord under general anaesthesia. A soft PVC-catheter (8 F) was introduced into the left branch of the portal vein and left there until portography, performed 2 days later for possible liver metastases (74). After completion of portography, a thin-walled Ödman-Ledin catheter (Mediplast, Denmark, ID/OD=1.4/2.2 mm), with the end curved about 80 degrees 3-4 cm from the tip and with 2 side-holes close to the tip, was introduced. It was placed in the main portal vein under fluoroscopic control and then sutured to the skin to prevent displacement. Details of the radiological procedure and its complications are given by Göthlin et al (74). All portal catheters were kept patent by slow continuous infusion of heparinized saline. The day after portal catheterization the patient resumed his preoperative routine, moved about at will in the ward and was on an ordinary hospital diet.

Experimental Procedures

In patients with portal catheters, the investigation was started the day after portography, i.e., 3 days after the introduction of the catheter. All patients fasted overnight. One hour before the beginning of the investigation, the infusion of heparin was discontinued and peripheral catheters were inserted. A Stille infusion cannula (bore 1.15 mm) was placed in a radial artery for blood sampling, and another in a contralateral cubital vein, when the patient was to receive a peripheral infusion. In patients studied twice the second study was performed 1 day after the first in random

sequence. Throughout each experiment saline, or glucose, was infused at a rate of 1.6 ml/min by means of a peristaltic pump. The rate of infusion matched, or slightly exceeded, the rate of blood loss due to sampling. Patients without a portal catheter were given saline via the peripheral vein catheter; otherwise saline or glucose was always administered via the portal catheter. The glucose was infused at a rate of 80 or 200 mg h⁻¹ kg⁻¹ and then the pretest periods were extended respectively 90 and 120 min to induce and maintain steady levels of glucose and insulin for about 30 min before the infusion of insulin (I). On the average, the infusions of glucose raised the arterial blood glucose levels by approximately 0.9 and 2.2 mmol/l, respectively, and the plasma insulin levels by about 4 and 22 µU/ml. During glucose infusion, portal blood glucose concentration was about 0.4 and 1.0 mmol/l higher than the arterial value (II).

Insulin Actrapid (monocomponent porcine insulin at neutral pH; Novo A/S, Copenhagen), diluted in 10 ml of saline, was infused manually at a constant rate for 2.5 min, after which the catheter was immediately flushed with 5 ml of saline. The intravenous glucose tolerance test (IVGTT) was begun 60 - 90 min after start of the insulin infusion. Twenty-five grams of glucose, dissolved in 100 ml of water, was given into a peripheral vein during 2 min. In the patients receiving continuous glucose infusion, the latter was continued throughout the IVGTT.

Three and 4 samples (2 ml) of arterial, and sometimes also portal, blood were taken during steady-state normo- and hyperglycemia, respectively. After the brief infusions of insulin, or glucose (IVGTT), blood was sampled for 60 - 90 min and at frequent intervals during the first 20 min. Portal samples were collected 10 s after the corresponding arterial ones. The contents of catheter dead space was always discarded immediately before sampling, which started at the specified time and took about 5 s. No anticoagulant was present in catheters and syringes used for sampling.

Analytical Methods

EDTA was added to sampling tubes as anticoagulant. Blood samples of 0.1 ml were immediately deproteinized for glucose determination. Plasma was separated rapidly and stored at -20°C until determination of insulin, C-peptide and FFA.

Glucose was measured in triplicate with a glucose oxidase method (75). The intra-assay coefficient of variation within the triplicate assays was 2.5 % at a level of 3.70 mmol/l and varied only slightly over the range of blood glucose concentrations studied.

Total insulin immunoreactivity was determined in triplicate with ethanol precipitation of the antibody-bound radioactivity (76). The antiserum reacts with proinsulin and cannot distinguish between endogenous human and exogenous porcine insulin. The standards were dilutions of human insulin (Novo Research Institute, Copenhagen). The stability of the assay was continuously checked on pools of human plasma included in every individual assay. Intra-assay precision within the triplicate determinations (coefficient of variation, mean) was 3.9 % for 97.0 μ U/ml, 7.0 % for 22.8 μ U/ml, 12.9 % for 8.1 μ U/ml and 49.8 % for 2.5 μ U/ml. The same parameters for between-assay reproducibility were 6.7 % for 97.0 μ U/ml, 9.3 % for 22.8 μ U/ml, 21.0 % for 8.1 μ U/ml and 68.0 % for 2.4 μ U/ml.

The C-peptide was assayed in triplicate according to Heding (77), reagents being purchased from Novo Research Institute, Copenhagen. The sensitivity of the C-peptide assay was about 0.05 pmol/ml and the intra-assay coefficient of variation within the triplicate assays was 17 %. Proinsulin contributes less than 3 % to the measured C-peptide.

FFA were determined according to Trout et al (78). With this method the titrated extract contains less than 0.5 % of the plasma lactate (78). The intra-assay coefficient of variation for FFA was 2 %, determined from 12 consecutive assays with tenfold determinations of a plasma sample of 420 μ mol/l.

In order to minimize analytical variation, samples from one patient were usually determined in a single assay run.

Insulin Adsorption

Actrapid insulin was iodinated with a substitution of 0.25 g-atom 125 I/mol insulin using the lactoperoxidase method (79). The labelled insulin was added to the stock solution of unlabelled insulin and the loss of iodinated insulin was determined. The concentration of the iodinated insulin was approximately 0.5 % that of carrier insulin. The loss to syringes and needles was determined by placing them in a well-type scintillation counter during and after use, whereas the loss to catheters was determined by the same procedure before and after use. All dilution and infusion procedures were identical with those of the experiments proper.

The adsorption losses decreased from 16 to 14 % with increasing dose. The catheters adsorbed 1.5-2 %. In papers I, II and IV the uncorrected doses are given in the text. Adsorption losses were taken into account in the calculations.

Calculations

Experimental data are denoted by the following symbols: $D(\mu\text{U/kg})$, dose of insulin; $I_a(t)(\mu\text{U/ml})$, incremental plasma insulin concentration in arterial blood after infusion of insulin, i.e., value above the pretest baseline; $I_{po}(t)(\mu\text{U/ml})$, incremental plasma insulin concentration in portal blood after infusion of insulin; $I_a^{ss}(\mu\text{U/ml})$, plasma concentration of (endogenous) arterial insulin in the steady-state; $I_{po}^{ss}(\mu\text{U/ml})$, plasma concentration of (endogenous) portal insulin in the steady-state; $APD(t)(\mu\text{U/ml})$, arterial-portal concentration difference. The superscripts v and $\wedge$ denote data from respectively the portal and the peripheral routes of infusion.

Insulin kinetics after infusion of insulin were studied at insulin levels above the pretest baseline. It was thus assumed that the endogenous insulin concentration remained unchanged throughout the clearance of insulin.

The plasma clearance rate (PCR, $\text{ml min}^{-1} \text{ kg}^{-1}$) was calculated as

$$\text{PCR} = D / \int_0^{\infty} I_a(t) dt \quad (1)$$

and is a measure of the average net clearance rate that is independent of the complexity of the disappearance (80-81). From a strictly formal point of view, the curve integral approach is generally not totally "model independent". The reason is that calculation of the tail area to infinity requires an assumption about the form of the final part of the curve. However, it is well-known that a monotonically decreasing function can be well approximated by an exponential even though the governing function may be of a different form. It should be observed that after portal infusion of insulin the first pass hepatic uptake is treated as an instantaneous loss (see Fig. 1) and that PCR includes the corresponding clearance rate.

Further characterization requires some type of model and thus additional assumptions. The methods described here - mean transit time and compartmental analyses - assume a linear and stationary insulin outflow mechanism. The mean transit time analysis is "noncompartmental" in the sense that it is valid whatever the compartmental structure (82, 83). As in the calculation of PCR one must, however, make an assumption about the final component of the curve. The corresponding model is often called a minimum-assumptions, or a noncompartmental, model (58, 82).

The mean transit time, $\bar{t}$ (min), was calculated as

$$\bar{t} = \left(\int_0^{\infty} t \cdot I_a(t) dt / \int_0^{\infty} I_a(t) dt - T/2 \right) \quad (2)$$

where correction is made for the $T = 2.5$ min insulin infusion (84, 85). Introduce the total apparent distribution volume, ADV (ml/kg), and the fractional disappearance rate, FDR (min^{-1}). Then:

$$\text{ADV} = \text{PCR} \cdot \bar{t} = \text{PCR}/\text{FDR} \quad (3)$$

ADV and $\bar{t}$ refer to molecules within and returning to the plasma pool (82, 83). As a consequence, the size of ADV ($\bar{t}$) depends not only on the physical distribution space and the concentration gradients between intra- and extravascular pools (ADV is plasma-equivalent), but also on the rates of exit from, and reentry to, the plasma pool. It follows that the greater the rate of degradation from extravascular sites the smaller the ADV. It is clear that ADV is a minimum volume and that ADV will approach the plasma volume when there is no reentry (cf. Appendix).

The integrals in equations 1-2 were calculated by the trapezoidal rule and exponential extrapolation of the tail of the curves. In each case, the exponential for extrapolation was given by the selected order of compartmental model (see below).

Assuming that the mean transit time for molecules escaping the first pass hepatic uptake of insulin is equal to that for peripherally infused molecules, the hepatic mean transit time, $t_h(\text{min})$, is given by (84)

$$\bar{t}_h = (\bar{t})^V - (\bar{t})^A \quad (4)$$

The kinetic study was focussed on the compartmental analysis because of its common use and its considerable potentiality. In this analysis molecules entering the systemic circulation (Fig. 1) were fitted both to a two-compartment model, with irreversible loss from the extravascular compartment, and a monocompartment model. After portal insulin infusion, the first pass hepatic uptake of insulin was treated as an instantaneous loss, whereas the hepatic uptake of post-hepatic¹ (recirculating) molecules as well as that of peripherally infused molecules was lumped together with the uptake in peripheral tissues. The differential equations, representing rates of change of concentration in each compartment, were:

¹ The term posthepatic denotes the insulin escaping the first pass hepatic uptake after portal infusion.

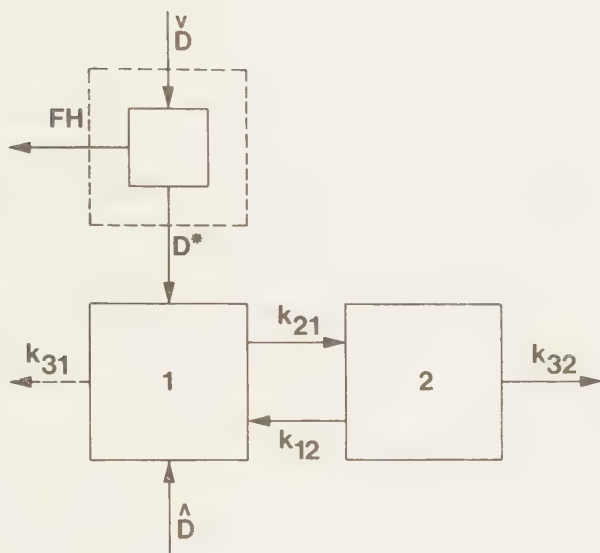


Fig. 1. Compartmental model for portal and peripheral infusion of insulin ($\bar{D}$, portal dose; $\hat{D}$, peripheral dose). After portal infusion, the first pass fractional hepatic uptake is designated FH . The first pass hepatic uptake is treated as an instantaneous loss meaning that $(1 - FH)\bar{D} = D^*$ (posthepatic dose) reaches the systemic circulation. The hepatic removal of posthepatic (recirculating), as well as of peripherally administered, insulin is lumped together with the removal in other tissues. For molecules entering the systemic circulation, flow between intravascular (1) and extravascular (2) compartments has rate constants k_{21} and k_{12} and the irreversible loss has rate constant k_{32} (or k_{31}).

$$d I_1(t)/dt = -k_{21} \cdot I_1(t) + V_2/V_1 \cdot k_{12} \cdot I_2(t) + B_0 \cdot U(t)$$

$$d I_2(t)/dt = -(k_{12} + k_{32}) \cdot I_2(t) + V_1/V_2 \cdot k_{21} \cdot I_1(t)$$

$$U(t) = 1 \text{ for } 0 \leq t \leq 2.5$$

$$= 0 \text{ for } 2.5 < t \quad (5)$$

The k_{ij} 's are rate constants, $I_1(t)$ ($=I_a(t)$) and $I_2(t)$ are the insulin concentrations in plasma and extravascular compartments, respectively, V_1 ($=APV$, apparent plasma volume) and V_2 the corresponding volumes and B_0 the computer-fitted infusion rate in $\mu U/min$ per ml of APV . $U(t)$ is a pulse of unit height representing the insulin infusion. In the mono-compartment model, k_{12} is zero. The fitting to experimental data points was started at 4 min, at which time intravascular mixing was assumed to be complete (86, 87).

The average net fractional transfer rate constant from plasma, $k_{e1}(\text{min}^{-1})$, is defined as PCR/APV . Following peripheral infusion of insulin, APV might be assumed to equal plasma volume. Following portal infusion, however, the size of PCR is influenced by the unknown first pass hepatic uptake. But the relationship $k_{e1} = k_{21} \cdot k_{32}/(k_{12} + k_{32})$ (for derivation, see Appendix) can be used to estimate k_{e1} without estimating APV . APV was then estimated from

$$APV = PCR/k_{e1} \quad (6)$$

The method of "peeling" was used to obtain initial estimates of the parameters in the two-compartment model. Final parameter values (B_0 , k_{12} , k_{21} , k_{32}) were determined from experimental data points with the nonlinear least squares program LISPID (88) on a Univac 1108 computer. In the least squares fitting procedure, the differences between model and experimental data were weighted with the inverse of the combined variance of the measurement error and (subtracted) pretest insulin levels. An example of the computations is given in Fig. 2 and Table 1. The infusion illustrated was considered representative of a two-compartment case (infusion 28 b, Table 5 in paper I).

The information content of the mono- and two-compartment models was evaluated by the minimum AIC (Akaike's information criterion) estimation (89). Generally speaking, for models with different degrees of complexity and with a similar degree of fit, AIC will choose the one that has the minimum number of free parameters. At the same time, AIC will choose the model that gives the most reliable description of a repeated identical experiment. The influence of the assay error on the deviations from a monoexponential

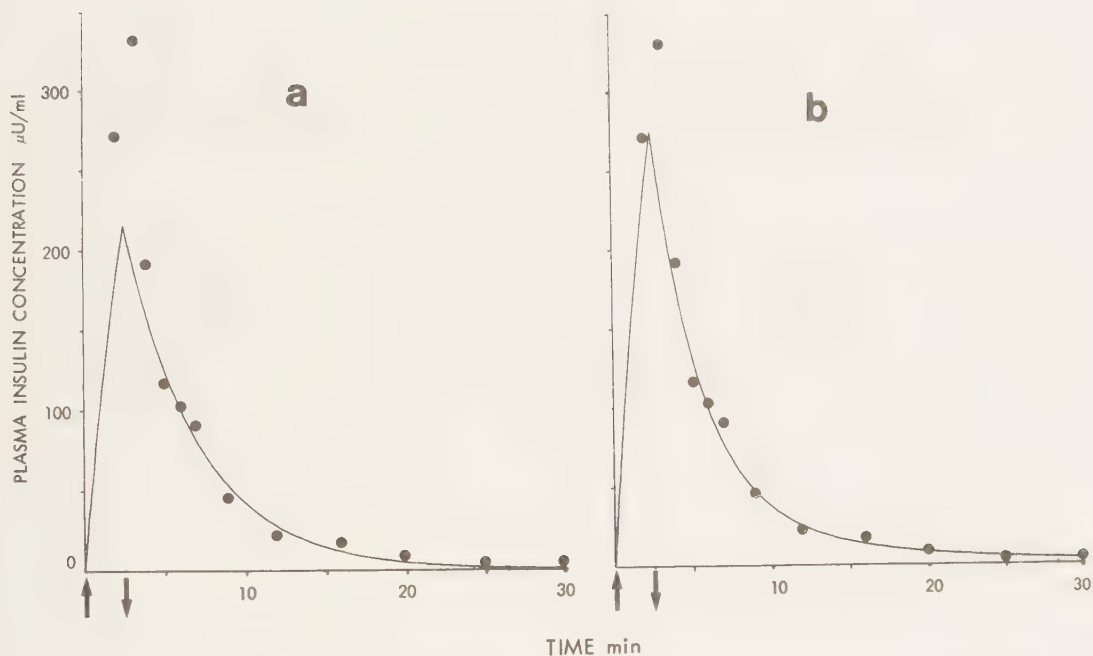


Fig. 2. Computer fit to a data set for which the two-compartment model might be valid. A peripheral infusion of 16.6 mU/kg insulin was given from 0 to 2.5 min. a) mono-compartment match b) two-compartment match.

Table 1. Numerical values of the observed (I_{obs}) and the computer-estimated (I_{est}) arterial plasma insulin concentration, the residual (ϵ) and the standard deviation (SD) of the measurement error for the infusion shown in Fig. 2.

Time (min)	Monocompartment match			Two-compartment match		
	I_{obs} (μ U/ml)	I_{est} (μ U/ml)	ϵ (μ U/ml)	I_{est} (μ U/ml)	ϵ (μ U/ml)	SD (μ U/ml)
4	192	155.6	36.4	176.9	15.1	17.4
5	117	125.1	-8.1	132.9	-15.9	9.0
6	103	100.6	2.4	100.7	2.3	7.6
7	91	80.9	10.1	77.2	13.8	6.7
9	45	52.3	-7.3	47.0	-2.0	3.9
12	22	27.2	-5.2	24.9	-2.9	2.7
16	16	11.4	4.6	13.2	2.8	2.4
20	9	4.8	4.2	8.3	0.7	2.0
25	3.5	1.6	1.9	5.3	-1.8	1.7
30	4.5	0.5	4.0	3.5	1.0	1.8

Weighting was performed with the inverse of SD^2 . The data represent infusion no. 28 b (see Table 5, paper I).

decline was tested by simple statistical analysis (I).

The two-compartment model was preferred to the monocompartment model when all the following requirements were met: 1) It had the lower AIC value. 2) It did not give negative or very large (infinite) k_{ij} values and/or positive or complex roots of the differential equation 5. 3) Its APV value was not below 20 ml/kg (90). Considering the widely different estimates of hepatic insulin uptake (52-64), a value of 45 ml/kg was chosen for APV. (The experimental design was such that if k_{el} had been correctly estimated, then APV should equal the value of plasma volume.) 4) It gave an estimation of APV that was lower than ADV. As shown in the Appendix, $APV = ADV / (1 + k_{12} \cdot k_{21} / (k_{12} + k_{32})^2)$.

The first pass fractional hepatic uptake of insulin, FH, was calculated from

$$FH = 1 - \hat{APV} / \bar{Y}_{PV} \quad (7)$$

For derivation, see paper II.

Assuming that there is no net exchange of insulin across the extrahepatic and extrapancreatic splanchnic bed, PCR_{ss} ($ml \min^{-1} kg^{-1}$), the plasma clearance rate of endogenous insulin in the steady-state, was calculated as (V)

$$PCR_{ss} = PPF (I_{po}^{ss} / I_a^{ss} - 1) \quad (8)$$

and $\bar{Y}_{ss}$ ($\mu U \min^{-1} kg^{-1}$), the pancreatic secretion rate in the steady-state, as

$$\bar{Y}_{ss} = PPF (I_{po}^{ss} - I_a^{ss}) \quad (9)$$

PPF is the portal plasma flow in $ml \min^{-1} kg^{-1}$ and is assumed to have a value of 10 (91-93).

In the steady-state, exchange across the extrahepatic splanchnic bed (ESB) was estimated from the paired arterial-portal differences (APD:s), assuming that flow, arterial concentration and exchange were constant (94). In the non steady-state, i.e., after infusion of insulin, exchange across ESB was estimated by the integration of APD:s up to the time at which the portal insulin concentration had returned to its pretest value, assuming that portal plasma flow was constant. Integration was trapezoidal. During the IVGTT, integration of the portal and arterial glucose curves involved the whole experimental period and included exponential extrapolation of the tail areas. After administration of insulin or glucose changes in APD were estimated after subtraction of the pretest APD value.

In order to estimate the effect of insulin on the ESB exchange of glucose and FFA also after portal insulin administration we used SER ($\mu\text{U/ml}$), simulated early insulin response, calculated as

$$\text{SER} = k_{el} \cdot \int_0^{\infty} I_a(t) dt \quad (10)$$

SER represents the total net insulin rise that would have occurred in the systemic circulation if all the insulin administered had remained in the plasma pool. It is an estimate of the peripheral or posthepatic dose, normalized by the plasma volume. By combining equations 1, 6 and 7 it can be shown that SER equals $\dot{D}/\text{APV}$ and $\dot{D}(1-\text{FH})\text{APV}$. The maximum rises in arterial plasma insulin were somewhat smaller than the SER values.

Insulin sensitivity was estimated from the effect of insulin on arterial glucose or FFA. It was calculated as the decrement from the pretest level to the mean of the lowest two consecutive concentrations and was expressed as percentual change from the pretest level. The k_G value ($\%/min$) of the IVGTT was calculated as proposed by O'Sullivan et al (95).

Only doses of 5 and 10 mU/kg gave insulin concentrations clearly within the physiologic range (I) and are referred to as "physiologic".

Statistical Methods

Parametric tests were used when the samples studied were large and their distributions, sometimes only after log transformation, appeared "normal". They were also used in those cases where non-parametric tests were not available and the distribution did not deviate significantly from the normal, as judged from the χ^2 -test.

Comments on Methods

For obvious reasons the portal studies were carried out on patients with malignant disease and after catheterization of the portal vein. But neither the disease nor the catheterization had any demonstrable effect on the variables studied (IV). The metabolic effects of operation and anaesthesia appear proportional to the severity of the trauma (96, 97). Considering the slight decrease in insulin sensitivity and the unchanged k_G values after inguinal herniorrhaphy, it is unlikely that the much less traumatizing portal catheterization should induce any significant impairment in these variables. As for the kinetics of insulin, inguinal herniorrhaphy was not accompanied by any change at all.

Before the experiments heparin was infused via the portal

catheter to prevent clotting in and around it. When administered in larger amounts, heparin raises FFA levels, inhibits glucose-stimulated insulin secretion and lowers tolerance to glucose (98). Also operation raises FFA levels (99). The combined effect of heparin and portal catheterization was estimated by comparing the fasting FFA concentrations in portally catheterized patients (n=31) with those in patients who had not been operated upon or received heparin (n=10). The FFA levels were respectively 350 ± 84 and 395 ± 157 $\mu\text{mol/l}$ (mean $\pm$ SD) (Mann-Whitney test, $P > 0.05$). It should be noted that the half-life of heparin is about 1 h, or less, after a dose as small as ours (100). Heparin does not affect the insulin radioimmunoassay used by us (101).

The removal and action of porcine insulin was considered representative of that of human insulin in man. The evidence is that it crosses vascular membranes at the same rate as human insulin in man (102) and that des-Ala^{B30} has full biological activity and presumably also full binding affinity to the insulin receptor(s) (103). Human and porcine insulins differ only in the amino acid situated in the B30 position.

A significant degree of incomplete mixing of exogenous insulin in the portal vein appears unlikely. The bent catheter tip with side-holes covered a major part of the transverse section of the portal vein and the insulin was infused in minute jets at right angles to the stream of blood flow. On the average, portal sampling seems to give representative values (55, 69, 92, 104) though they sometimes show a relatively wide random variation (55). During the IVGTT we may have missed the insulin peak in the portal vein, but the results obtained with more frequent sampling do not suggest that this had any major influence (104, 105).

To study the plasma disappearance of insulin we gave a brief infusion, which has the following advantages and disadvantages. The continuous infusion method gives results that are easier to interpret, but the information content is rather low and it is not self-evident that the results apply to conditions other than the steady-state (80). To obtain interpretable plasma curves with the instant injection method, the initial concentrations must be nonphysiologic. The latter problem can be obviated by the use of radioiodinated insulin, but then other difficulties arise (see below). A short infusion provides a physiologic delivery rate while preserving the possibility of estimating fractional transfer rate constants and distribution volumes. The main disadvantage of a brief infusion is that the initial part of the plasma insulin curve is difficult to characterize, especially when disappearance is rapid. The uncertainty of the initial area is, however, less than after instant injection (81).

Another advantage of the method of brief infusion, or instant injection, is that it gives information about the primary action of insulin. With the continuous infusion method one measures a mixture of primary and secondary, compensatory changes. In order to study the primary effect of physiologic elevations of plasma insulin it is, again, preferable to use a short infusion instead of an instant injection. It should be noted that cells respond maximally to insulin when only a small fraction of available receptor sites are occupied (39). With respect to relationships between insulin disappearance and insulin action, we could, however, not find any differences between physiologic and larger doses.

Labelled insulin is more suitable for modelling because it can be measured more precisely. But the validity of the results with labelled insulins has been seriously questioned, both with respect to disappearance and biological activity (9, 14, 15, 23, 106-108). Tracer amounts of iodinated insulin require a degree of iodination that influences the removal (9, 14, 108), and do not allow simultaneous estimation of the effect of insulin. "Lightly" labelled insulins require amounts of insulin that are comparable to ours. With these compounds the system is thus not in the steady-state and may be nonlinear and/or nonstationary (I), with a consequent loss of the theoretical advantage with labelled insulin. It should be remembered that the insulin molecule cannot be labelled with less than one atom of iodine, and that even lightly labelled insulin behaves in a manner different from unlabelled insulin (9, 14).

In the calculations the most important and critical assumption is that insulin secretion remains unchanged throughout the plasma clearance of exogenous insulin. Whether insulin can directly inhibit its own secretion is a controversial question (109-121). The striking lack of agreement between the findings, especially in vitro, and the fact that the concentrations of insulin commonly used to demonstrate direct feed-back are very high indicate that any direct inhibitory effect of insulin is at most slight. Studies in vivo show one consistent finding, viz. a depression of glucose-stimulated insulin release after a long preceding infusion of insulin or insulin and glucose (109, 113, 114). Such an effect is probably caused by changes secondary to the increased insulinization of tissues and not directly by insulin itself. Our own studies of the temporal relationships between insulin, C-peptide and glucose (III) indicate that insulin does not immediately inhibit its own release and that it is the glucose decrease that is responsible for the decreased secretion rate of insulin. The above considerations imply that exogenous insulin had no direct effect on insulin release during and after the brief infusions of insulin in our experiments.

The decrease in the rate of insulin secretion that follows administration of insulin can instead be ascribed to the lowering of the blood glucose (116, 122-124). We could not correct for a possible effect of the glucose decrement on the baseline insulin level because of the variability and inconsistency of the C-peptide concentrations, and because of the absence of change in APD even when arterial and portal concentrations decreased (III). On the average, C-peptide did not start to fall before the arterial insulin concentration had returned to its pretest value, which was the end-point of the measurement of insulin clearance. The decrease in C-peptide seemed to vary with insulin dose and arterial blood glucose decrement, though C-peptide decreased only when the blood glucose decrement was 15 % or more. It did not decrease after a dose of 8 mU/kg. In paper I the "equilibrium" arterial insulin concentrations after the presumed clearance of insulin were compared with those recorded before infusion of insulin. The depression of the arterial insulin level was small, unrelated to insulin dose and only very weakly related to the blood glucose decrement. The importance of these findings is, however, doubtful since the post-clearance values may be influenced by reentry to the plasma pool. On the other hand, the calculation of PCR stopped as soon as the pretest baseline concentration was reached, which means that a "false" depression of the insulin level may be obtained owing to separation of randomly distributed data points at the tail end of the curve. Attempts to estimate the degree of depression of the baseline insulin level with a Derivimeter (125) were unsuccessful because of the irregularity of the curves and the uncertainty of the high derivatives. In other studies the hypoglycemia has been much more sustained and pronounced (118, 122-124).

Suppression of endogenous release is presumably gradual in time. Correction for this, together with more frequent sampling late in the experiments may result in unchanged, or even worse, estimation difficulties because of the uncertain determination of the low insulin concentrations and the uncertainty of the baseline level. Sherwin et al. (20, 126) used a glucose clamp to avoid fluctuation of the blood glucose level and supposedly also of the endogenous insulin secretion rate. But the interpretation of their data is open to question. After an initial blood glucose descent, comparable to that in our experiments(!), there was a subsequent rise in blood glucose to levels slightly exceeding the basal and accompanied by a rise in the arterial plasma insulin concentrations (20, 126). Thus, a perfect clamp was not obtained and it is therefore possible that "the slowly equilibrating (third) compartment", i.e., the final exponential in the decay curve in refs. 20 and 126 reflects increased secretion from the endogenous "pancreatic pool".

Summing up, there is no evidence that the pretest insulin level was a proper baseline throughout the clearance of exogenous insulin. The subtraction of the pretest level should give fairly correct results for the physiologic insulin doses. After larger doses the simple subtraction was probably not correct. But the quantitative error appears to be small since computations performed under the assumption that the endogenous arterial insulin level was lowered by 1 or 2 $\mu\text{U/ml}$ induced only small increases in PCR (I).

As discussed in paper I, the assumption of linearity and stationarity was not correct. From a strictly formal point of view our models were thus inappropriate. Apart from the fact that we could not distinguish a more appropriate model from our data, we believe that the analysis of these models, including analysis of factors limiting them, is justifiable. Mean transit time and compartmental analyses are frequently used and they have given interesting results, both for unlabelled (20) and labelled (13, 16, 58) insulins, with doses comparable to ours and with doses that also others (9, 10, 14, 22, 107) have found to be cleared in a dose-dependent way. Administration of lightly labelled insulins is accompanied by relatively large amounts of unlabelled insulin (13, 58). In our study the noncompartmental and compartmental analyses comprised only the insulin that entered the systemic circulation. After portal infusion of insulin, the dose-variation of PCR was due mainly to a dose-dependent variation in FH. Furthermore, part of the dose-variation of k_{el} and APV was due to poor parameter accuracy (I). But after the largest doses there was a real decrease in clearance of systemic insulin, as demonstrated for posthepatic insulin (II) and for peripherally introduced insulin at hyperglycemia (I). Since the data indicate an inaccurately low estimation of the highest insulin concentrations, there may have been a slight decrease in PCR with increasing dose following peripheral infusions at normoglycemia, too (I).

Despite its limitations the compartmental analysis proved valuable in the estimation of FH (equation 7). As demonstrated by us (II), and by others (84), FH may be calculated as

$$FH = 1 - \frac{\hat{V}}{PCR/PCR} \quad (11)$$

Equation 11 is inferior to equation 7 because it is less reproducible and because it gives false results when the clearance rates of peripherally administered and posthepatic insulin differ systematically from each other. The shortcomings of equation 11 are due to the assumption that the clearance rate of systemic insulin after portal infusion is identical with that after peripheral infusion in another experiment (II). Calculation based on PCR has a low reproducibility because these clearance rates are subject to

every detail of the experiments, e.g., to factors such as changes in the distribution and rate of blood flow and changes in uptake in various tissues, which may vary from one occasion to another in a given individual. It will underestimate FH if first pass occupancy of hepatic insulin receptors decreases the clearance rate of posthepatic (systemic) insulin (II).

Calculation based on APV (equation 7) avoids the assumption of identical clearance rates for the insulin reaching the systemic circulation after the two infusions. Instead it assumes that systemic insulin reaches the same volume of plasma, here estimated as APV (II). It has been demonstrated (127) that the plasma volume varies but little from day to day in a normal subject. Assuming that the plasma volume is the same in the two experiments, the amount of the portally infused insulin that reaches the systemic circulation is calculated. FH is then determined by comparison of the amounts of insulin given to, and transmitted through, the liver, which is equivalent to comparison of the APV values after infusion into respectively the portal and a peripheral vein (equation 7). The better reproducibility of APV than PCR, as well as the better reproducibility with equation 7 than with equation 11, is explained by the covariation of PCR and k_{el} (I). In other words, a small PCR value, produced by a large insulin area, is accompanied by a relatively small k_{el} value which gives a relatively small variation in APV (equation 6).

The calculation of FH based on APV is not without problems. Thus, APV increased with the dose (I, II), presumably due to inability of the compartmental analysis to identify properly a small reentry of insulin from the extravascular compartment(s) with a consequent effect on the k_{el} value. Despite the rather poor accuracy in the estimation of k_{el} after the larger doses, the sensitivity of the compartmental analysis was sufficient to introduce only a slight degree of uncertainty in k_{el} as well as values of APV that did not differ between infusions fitted with one and two exponentials in this dose range (I). The variation of APV with dose implies that the doses should be matched in such a way that the arterial insulin concentrations are similar after the two routes of infusion. But it is noteworthy that the correlation between $\bar{D}$ and APV was mainly a result of different APV values following physiologic and larger doses, and that APV did not vary with doses exceeding 10 mU/kg (I). It may thus be sufficient to get either only physiologic or only supraphysiologic arterial concentrations via the two routes. It may be difficult to achieve similar concentrations when the hepatic uptake is unknown.

Summing up, our approach of calculating FH does not require

determination of hepatic or posthepatic blood flows nor the determination of plasma insulin levels in the portal or a hepatic vein. It should be suitable for determining uptake in any organ where it is difficult to obtain representative samples from afferent and/or efferent blood and/or where determination of blood flow is inconvenient.

In the two-compartment model the site of irreversible loss (catabolism) was assumed to be extravascular, but the solution fits identically well with assumed catabolism from the plasma pool only (k_{31} in Fig. 1). The use of the alternative exit would give identical values for k_{e1} ($= k_{31}$) and would not influence the validity of the comments on the compartmental analysis (I).

The effect of insulin on arterial glucose, or FFA, was expressed as maximum decrease and not as total decremental area in order to distinguish the primary action from late, compensatory changes. However, both methods of calculation gave similar results because the maximum decrease and the total decremental area were closely correlated. The rate constant for plasma glucose disappearance, k_{IVITT} (min^{-1}), is another way of estimating sensitivity to insulin (17, 34, 45). On the average, k_{IVITT} corresponded well to the blood glucose decrement. But we felt reluctant to use k_{IVITT} because of the high degree of arbitrariness in choosing the start and end points in the calculations. This difficulty is obvious from the blood glucose curves shown in papers I-V. Owing to variability and delays, or plateaus, in the blood glucose response it is also questionable whether the assumption of an exponential decrease is (always) justified. The action of insulin on blood glucose, or plasma FFA, was expressed in per cent of the pretest value since this is related to the response (128).

Exchange across the ESB was estimated with the area method, which has the potential draw-back that rapid time-bound changes, such as initial uptake and subsequent release, may escape demonstration. But our data after insulin, or glucose, did not suggest bidirectional exchange across ESB during the individual experiments. After infusion of insulin it was assumed that portal flow was constant, which should be a correct assumption when the blood glucose decrements are as small as in our experiments (55, 69, 71, 73, 91). Insulin itself does not affect portal blood flow (55, 69, 71, 73, 91). Accordingly, the demonstration of uptake, or release, is a true demonstration of the direction of the average net exchange. On the other hand, our studies cannot exactly quantitate this exchange because we did not measure blood flow. The total uptake and the fractional extraction ratio of insulin across the ESB were estimated only in order to give an idea of the size of these figures (III). They are

uncertain because of the uncertainty of the APD areas and because we had to rely on published values for portal flow. Furthermore, it was assumed that the fractional extraction ratio was constant. After the injection of glucose there is probably a slight and transient increase in portal blood flow early during the IVGTT (92, 129). However, since the APD values did not change sign during the experiments, the area method can, again, disclose whether or not there was uptake or release. It should be noted that correct quantification of the extrahepatic splanchnic uptake of glucose during the IVGTT requires that APD:s as well as portal plasma flows be measured at frequent intervals throughout the clearance of exogenous glucose (94).

Demonstration of a possible uptake of insulin in the ESB is particularly liable to be erroneous because the immunoassay cannot distinguish the endogenous insulin from the exogenous insulin and because the endogenous release may be suppressed. It was therefore necessary to determine C-peptide levels. These measurements showed that insulin secretion decreased, but that it did not start to do so before the portal insulin concentration had returned to its pretest value, which was the end-point of the measurements of insulin APD. Thus, the calculated areas for insulin APD were probably only slightly influenced by a decrease in the rate of insulin secretion.

The $\overline{PCR}_{ss}$, and $\overline{SR}_{ss}$, values (V) were calculated with a PPF value derived from the literature. This was done mainly in order to show what the terms $(I_{po}^{ss}/I_a^{ss}-1)$ and $(I_{po}-I_a)^{ss}$ represent. As in the calculations of nonhepatic splanchnic exchange, a constant PPF value should not give correlations that are not obtained if flow measurements are included. Conversely, it is more likely that the use of the same PPF value for all patients underestimates the true relationships. The validity of correlating arterial concentrations and APD:s (III, V) without determining blood flow is supported by the following. Inclusion of hepatic blood flow in the multiple regression equation of the arterial-hepatic venous differences for FFA (dependent variable) versus arterial FFA and arterial lactate did not decrease the mean square of the deviations (130). In other words, variation in arterial FFA and lactate accounted for almost all of the variation in splanchnic exchange, whereas hepatic blood flow did not contribute at all. This may be explained by a small interindividual variation in hepatic blood flow and a relatively small precision of its measurement (131), which thus cannot reduce the "random variation". Portal blood flow measurements presumably have the same variability.

The (mere) existence of a correlation does not give any evidence as to the cause and effect between the variables. Accordingly, the conclusions warranted or suggested by the

correlation analyses were based on the experimental circumstances as well as present and prior knowledge from other sources. A related problem is that variables can vary together, making it difficult to single out the importance of any particular one. For instance, it is possible that the association between relative body weight and PCR, or the absence of association between PCR and k_G , (IV) was due to the relations with one or more other variables, such as the fasting plasma insulin concentration. The multiple regression analysis was therefore used to assess the closeness of the association between two variables after elimination of the effects of the others.

The almost complete lack of correlations between the clinical variables and insulin kinetics (IV) may in part have been due to the rather narrow ranges of the clinical variables, but there may be other explanations as well. The regression and correlation analysis has the draw-back that some other (curved) form of relationship might have been better than the linear function used. (The lack of a demonstrable relationship does not exclude the existence of another kind of relationship.) But scatter diagrams did not indicate curved relationships and the "random" variation seen in most of them further suggested that factors other than those tested were important (IV).

In statistical tests there is always a risk of significance by chance, and it increases with the number of tests. A P value between 0.01 and 0.05 should therefore be interpreted with caution when many tests are carried out and the result is in conflict with other findings. In the Results section entitled "Relations between Insulin Kinetics and other Metabolic Variables" in paper IV, 28 statistical tests were performed between PCR or APV, on one hand, and various clinical variables, on the other. Formally, the probability is about 76 % that 1 test is significant by chance at the 5 % level of significance. However, the validity of the 2 significant correlations is enhanced by the fact that these correlations were consistent with trends in other correlations (data not shown) and by the fact that the plasma insulin removal parameters as well as the clinical variables are interrelated.

RESULTS WITH COMMENTS

Models for Insulin Disappearance

The plasma disappearance curves after brief infusions of insulin were analysed in 62 patients receiving 52 portal and 68 peripheral infusions (I). The PCR_{ss} values for endogenous insulin during steady-state normo- or hyperglycemia were estimated in 45 patients (V).

After intraportal infusion of insulin (4.2-43.0 mU/kg) at normoglycemia, PCR decreased progressively from 32 to 14 ml $min^{-1} kg^{-1}$ with increasing dose. After intraportal infusion of insulin (8.4-34.4 mU/kg) at hyperglycemia there was also a decrease, but this was not significant, in PCR with increasing dose. After peripheral infusion (4.2-25.5 mU/kg), PCR varied negatively with the dose at hyperglycemia, but not at normoglycemia (mean value 15 ml $min^{-1} kg^{-1}$). Regardless of route of insulin infusion or blood glucose level, the FDR ($= 1/\bar{t}$) and k_{el} values in respectively the mean transit time and compartmental analyses varied negatively with insulin dose. The distribution volumes varied negatively with the dose after peripheral infusion. Intra-assay variability was shown to have a profound influence on the shape of the curves.

The mean transit time analysis can reveal whether there is any detectable reentry of the infused insulin to the plasma pool, i.e., whether it is possible to define more than one compartment from the plasma insulin data, and whether the results of the compartmental analysis are reasonable (see Calculations and Appendix). FDR ($= 1/\bar{t}$) refers to net fractional removal rate from the total distribution volume (estimated as $\hat{ADV}$), whether it be the plasma volume (no reentry) or a larger volume (reentry), whereas k_{el} refers to net fractional transfer rate from the plasma compartment (estimated as $\hat{APV}$). In the monocompartment model $\hat{APV}$ should thus equal $\hat{ADV}$ and plasma volume. In the two-compartment model (with reentry) $\hat{ADV}$ should be larger than $\hat{APV}$, which should still equal plasma volume. After the two smallest, physiologic, doses $\hat{ADV}$ was rather close to $\hat{APV}$ and other estimates of plasma volume (90), and the monocompartment ($k_{12} = 0$) approximation seemed adequate for describing the disappearance of insulin in the systemic circulation. After larger doses there was a detectable reentry of insulin to the plasma pool as indicated by the increase in $\hat{ADV}$. But, as a rule, the compartmental analysis could not accurately determine $\hat{k}_{el}$ (and $\hat{APV}$) as demonstrated by the increase in $\hat{APV}$ and the fact that $\hat{APV}$ was not smaller than $\hat{ADV}$ (Table 3 in paper I). Other examples of the limited power of the compartmental analysis are given in paper I.

If ΔP_V had been assumed constant, k_{el} would not have decreased with increase in insulin dose at normoglycemia, suggesting that the dose-variation of k_{el} (and ΔP_V) was due only to poor parameter accuracy. But, as discussed, the results suggest that PCR was only apparently independent of dose after the two largest doses. At hyperglycemia PCR actually varied with the dose. After portal infusion of insulin, the major part of the dose-variation of PCR was due to a dose-dependent FH (II), and the clearance of posthepatic insulin could not be demonstrated to vary with the dose for doses up to and including 20 mU/kg (II). However, after larger doses there was a decrease in the clearance rate of systemic (posthepatic) insulin. Part of the dose-variation of k_{el} (and ΔP_V) thus appears to be a true reflection of a nonlinear removal of systemic insulin, whereas this variation seems to be due entirely to poor parameter accuracy after the three smallest doses. It follows that we could not demonstrate nonlinear clearance of systemic insulin within the physiologic range. This agrees with some (132, 133), and disagrees with other (22, 107), studies with unlabelled (22, 132) or labelled (107, 133) insulins, which suggests that the clearance of systemic insulin changes but little with the concentration within the physiologic range. It corroborates the assumption that a monocompartment model is fairly adequate in this situation. After portal infusion of insulin, ΔV and ΔP_V did not vary with the dose. The explanation is that the effect of a decreasing FH happened to be roughly counterbalanced by a corresponding decrease in FDR ($1/(t)$) or k_{el} .

The shortcomings of the noncompartmental and the compartmental models were caused mainly by the imprecision of the experimental data, in particular the imprecision of the immunoassay, and the difficulty to define a proper baseline concentration. In this respect, the nonlinearity demonstrated appeared to be of less importance. The profound influence of the intra-assay error on the shape of the curves, and thus on the estimated parameter values, was demonstrated by simple statistical analysis (I). It may also be predicted from the results of direct quantitative analysis of the effect of random data variation (134). Finally, the very rapid disappearance of insulin from plasma is an additional obstacle in modelling attempts.

The increase in ΔV with increasing dose is most likely explained by a decreased rate of irreversible loss in the extravascular compartment(s) (I). It does not seem compatible with saturation of intravascular sites and therefore suggests that at least part of the irreversible loss takes place in the extravascular pool(s). The data do not exclude that there is degradation from intravascular sites, too.

The contention that PCR predominantly reflects irreversible loss from plasma during the experimental period (0-60(-90) min) is supported by the findings of Sönksen et al. (22) that the disappearance curve of insulin after stopping of an infusion is similar to the disappearance curve obtained after instant injection. Yet it is possible that some insulin molecules return to the plasma pool at a later time (20, 135), meaning that a prolonged observation period would show that a fraction of the PCR value represents only a temporary loss. Thus, we cannot claim that our results after infusion of insulin describe insulin kinetics completely or that they are valid for all situations. But the results obtained for truly physiologic doses of insulin should be valid for plasma insulin disappearance during short periods of increased insulin secretion, such as ordinary 1 h IVGTT.

As far as we know, the study in paper I is the first to examine the influence of variation of dose and route of infusion as well as the influence of experimental data variation on attempts to model insulin kinetics. These factors are either ignored or handled in an arbitrary way and this is probably a major reason why there is such a wide divergency of opinion as regards the manner and rate of insulin disappearance (8-27). The very low $t_{1/2}$, 1.9 min, for systemic insulin has not been reported before simply because other workers have used larger doses. Another reason for divergency is the study of arbitrarily selected parts of the curves, i.e., of initial or late portions that appear straight in the semi-logarithmic plot. This, as well as the use of different doses, explains why $t_{1/2}$ has varied from 3 to 13 min (25, 15). Palumbo et al. (16) and Stimmler et al. (26) studied the whole disappearance curve after instant injection and concluded that compartmental analysis is not at all suitable. Our results suggest that it can give only limited information. Sherwin et al. (20) do not agree, but it should be remembered that the interpretation of their results is open to question.

So far we have not considered the results in the steady-state (V), which undoubtedly further complicate the picture. We found that PCR_{ss} (for endogenous insulin) was lower in the basal fasting state than during glucose infusion. At normoglycemia PCR_{ss} was much lower than the PCR values obtained after the brief infusion of insulin, whereas the PCR_{ss} and PCR values were similar during glucose infusion. In addition, there was no association between PCR_{ss} and PCR values within patients.

The observation that the clearance rate of insulin is lower in the basal unperturbed state than otherwise may be due to poor specificity of the immunoassay (V). But it also raises the possibility that clearance rates are different during

non steady- and steady-state conditions and that they differ between different steady states. The absence of correlations between PCR and PCR_{ss} might have been due to the uncertainty of both estimates. Another explanation is that some of the determinants of the plasma clearance rate of insulin shortly after insulin administration play only a minor role for the clearance rate in the steady-state(s). The above discrepancies corroborate the view that results obtained in the non steady-state can be applied only to (short) periods of increased secretion of insulin.

In conclusion, the information content and the interpretation of data are disturbed by the relatively serious data errors and the problems involved by perturbation of the basal steady-state. But until a better model has been developed, perhaps with "selectively" labelled insulin (107), it seems justifiable to use the monocompartment model for systemic insulin, provided the concentration is raised above the basal level and still is within the physiologic range.

Hepatic Uptake of Insulin

The first pass hepatic uptake of insulin was determined in 45 patients, 30 of whom were studied at normoglycemia and 15 at hyperglycemia (II). FH varied negatively with insulin dose at normoglycemia, decreasing about 50 % after a tenfold increase in dose (4.2-43 mU/kg), but it did not decrease significantly with the dose (8.4-34.4 mU/kg) at hyperglycemia. It was markedly less during glucose infusion than at normoglycemia. After small doses (4.2-8.4 mU/kg), giving portal insulin concentrations within the physiologic range, FH was 0.43 (4.2 mU/kg) and 0.40 (8.4 mU/kg) at normoglycemia and 0.16 (8.4 mU/kg) during glucose infusion. In no instance did it vary with the pretest endogenous portal, or arterial, insulin concentration. The endogenous insulin concentrations in portal and arterial plasma during the IVGTT strongly indicated a smaller hepatic uptake in this situation than at normoglycemia.

The most interesting finding is that moderate hyperglycemia decreased the (first pass) fractional hepatic uptake of insulin. This contrasts with the results, or conclusions, reported in most (52, 53, 56, 63), but not all (57, 64), previous studies in vivo. Studies in vitro on the effect of glucose are scanty and contradictory (136, 137). The decrease in FH with increasing dose is less surprising, and agrees with results obtained in vitro (35, 42-44, 138) and some obtained in vivo (9, 54, 59). But, again, it disagrees with the conclusions drawn from those studies in vivo where the rise in portal insulin was induced by glucose (52, 53, 56, 63). The prevailing concept is that the fractional hepatic uptake of insulin is unchanged, or increases, as blood

glucose and/or plasma insulin increases (e.g. refs. 20, 29, 30, 32, 123, 124, 139), and this view stems primarily from studies with hepatic vein sampling, the most commonly employed technique (9, 52, 54-56, 59-62, 64). This technique is, however, treacherous, and its results should be carefully interpreted.

Thus, the results obtained with the hepatic vein catheter seem to vary with the portal-arterial gradient of insulin. When the portal insulin level is raised above the arterial, by infusion of glucose (52, 56) or insulin (55), the fractional hepatic uptake increases (52, 56) or is essentially constant (55) as portal insulin increases. (There is a remarkably wide variation of fractional hepatic uptake in these studies and, at hyperglycemia, divergent findings have been reported once (64).) In contrast, when portal and arterial concentrations are similar, as after peripheral administration of insulin, there is a clear-cut negative relationship between plasma insulin concentration and fractional uptake in the liver (9, 54, 59). We believe that these discrepancies are explained by the findings of Sapirstein & Reininger (140) and Brauer et al. (141) that hepatic vein sampling is apt to overestimate the hepatic uptake in proportion to the gradient between portal and systemic levels. They (140, 141) found that a deeply placed hepatic vein catheter received blood originating mainly from the hepatic artery and that withdrawal of the catheter was accompanied by a gradually increasing admixture of blood from the inferior caval vein. There seemed to be no ideal position of the catheter and the results were highly variable (140). Species variation cannot account for the above discrepancies because all refer to studies in dogs. It is also noteworthy that calculation of the fractional hepatic uptake in the dog with equation 11 gives a much lower value (58) than hepatic vein sampling.

Whereas errors with the hepatic vein catheter thus may explain some of the conflicting results, they do not seem to explain the findings in two recent studies in dogs that pharmacological doses of glucagon (61) or arginine-cholecystokinin-pancreozymin (62) decrease the fractional hepatic uptake of insulin. Although the decrease in fractional liver uptake was accompanied by a large increase of blood glucose in both studies (!), these reports are important in emphasizing that the regulation of the hepatic uptake of insulin may be complex and that also factors other than glucose and insulin may play significant roles. (The data reported by Sun et al. (42) have the same implication.) It was suggested (61, 62) that increased pancreatic glucagon directly caused the decrease in hepatic insulin uptake, but this mechanism cannot explain a decrease in hepatic uptake during glucose infusion. Lack of an effect of increased pancreatic glucagon

is corroborated by findings in vitro (44, 136) and the finding that portal and peripheral glucagon infusions during the IVGTT give almost identical values for peripheral plasma insulin (142).

It has been reported that the fractional hepatic uptake of insulin increases when glucose is given by mouth, or stomach tube (53, 63). The conclusions were drawn from correlations between the portal minus peripheral insulin concentration, on one hand, and the simultaneous portal insulin concentration, on the other. But the portal-peripheral difference is essentially an estimate of the insulin release rate and not a direct measure of the hepatic uptake. It is natural that the insulin release rate and the portal insulin concentration vary with each other. Thus, these data (53, 63) can neither prove nor exclude that glucose transport across the intestinal wall causes the release of a factor that increases the hepatic clearance of insulin.

Our studies do not reveal whether the decrease in FH in response to glucose is a direct effect of glucose or a result of secondary changes. Whatever the mechanism, the results of the IVGTT suggest that rapid biochemical pathways are involved. The decrease in FH during glucose infusion was not due to an increase in portal blood flow (52, 67, 71), and the evidence is against the possibility that pretest occupancy or down regulation was responsible (II). The small portal-arterial gradients of insulin during the first few min of the IVGTT (Fig. 7 in paper II) were, at least partly, due to a transient increase in portal plasma flow (92, 104). Notably, these gradients as well as those in the studies of Röjdmärk et al. (61, 62) were so small as to suggest a release of previously bound insulin from the liver. However, such an effect of glucose has no solid experimental support (II).

The uptake of a comparatively small fraction of insulin in the liver during hyperglycemia, and a large one during normoglycemia, is consistent with the finding that the hepatic effect of insulin on blood glucose is smaller at hyper- than at normoglycemia (143), showing that hepatic uptake and effect of insulin vary with each other. It is consistent with the findings that a) the main part of exogenous glucose is taken up in peripheral tissues (144-146), b) glucose itself rather than insulin is the main determinant of net hepatic glucose balance provided there is a low constant hepatic supply of insulin (147, 148), and that glucose itself can inhibit net splanchnic glucose production (149, 150), c) human muscle tissue is quantitatively more important than the liver in storing glucose as glycogen for later use (151), and that insulin effects glucose uptake by muscle only when insulin concentrations are elevated clearly above the basal

level (150). It agrees with the metabolic requirements in the fasting and the nonfasting state also in other respects (65). Finally, it shows that increased glucose gives changes in peripheral plasma insulin that do not reflect changes in pancreatic secretion rate alone. This is true whether or not there is any direct effect of insulin itself on hepatic clearance.

It is tempting to speculate that the effect of glucose on the hepatic uptake of insulin holds also for diabetics. Suffice it here to mention that Frost et al. (10) found PCR to be comparatively small and nonsaturable in severe diabetics (with high fasting blood glucose levels) but to have a saturable component in normal fasting subjects. Since our findings (II, IV) indicate that the effects of insulin and glucose on PCR are predominantly effects on the hepatic uptake it is conceivable that the hepatic uptake of insulin was virtually absent in these diabetic patients.

Uptake of Insulin in the Extrahepatic Splanchnic Bed

The uptake of insulin in the ESB was estimated in 12 patients given peripheral infusions of insulin. By calculating SER, the effect of insulin on the extrahepatic splanchnic exchange of glucose and FFA was evaluated also in 13 patients receiving portal infusions of insulin (III). After the peripheral infusion of insulin a small amount of it was taken up in the ESB, and the integrated APD values for insulin varied with the integrated APD values for glucose. After portal and peripheral infusion of insulin there was an uptake of glucose across ESB, whereas the mean integrated APD value for FFA did not differ from zero. But the APD areas for FFA varied negatively with SER and negatively with the decrements of systemic blood glucose and plasma FFA.

The correlation between the balances of insulin and glucose across ESB corroborates that insulin was taken up in this region. But the extent of this uptake appears uncertain. In previous studies the fractional extraction ratio has been estimated to be 0 (69), 0.04 (59), 0.10-0.15 (70) and 0.38 (55) after administration of insulin. The first study was performed in sheep, the others in dogs. The value of 0.38 is unlikely high. Our own estimate of the fractional extraction ratio (see Appendix in III) is uncertain and, as discussed (III), may be too high. We suggest that after administration of insulin the fractional extraction ratio of insulin across ESB is in the range of 5-10(-15) %. As for the fractional extraction in the basal state, previous studies have indicated that it is 0 or so small as to be undeterminable (55, 69, 70). The explanation may be that an extraction of 5-10 % is indistinguishable at low insulin concentrations (below 50 μ U/ml). But it cannot be excluded that

the fractional extraction is different in the basal unperturbed state than otherwise (confer the difference between $\overline{PCR}$ and $\overline{PCR}_{SS}$ values at normoglycemia).

Insulin caused a net uptake of glucose in the ESB, whereas it had no effect on the ESB exchange of FFA when the results were pooled in the usual way. These findings agree rather well with previous results in dogs (71-73). But the pronounced interindividual variation in APD values for FFA suggested that mesenteric FFA metabolism was affected, and this was demonstrated by the correlations with the insulin action in the body as a whole. The direction of net exchange of FFA across the ESB indicates that small elevations of arterial plasma insulin, with small effects on systemic blood glucose and plasma FFA, caused a net uptake of FFA in the ESB, whereas large elevations of plasma insulin, with relatively large decrements of arterial glucose and FFA, were accompanied by a net release of FFA from this region. Insulin thus seems to be involved also in the regulation of FFA across the ESB though the net release is probably due to decreased (decreasing) blood glucose and plasma FFA levels, i.e., due to changes secondary to insulin action. As for the extrahepatic splanchnic exchange of glucose, a similar variation with insulin dose was not proven and could only, rather speculatively, be suggested from the data. It should be noted that uptake as well as release of glucose (73, 152) and FFA (130, 153, 154) across the ESB is consistent with observations in vivo (73, 130, 154) and in vitro (152, 153).

Contrary to the general belief, the study shows that total and hepatic splanchnic exchange of glucose and FFA cannot generally be equated. The effect of insulin on arterial-hepatic venous differences should therefore be interpreted with caution.

Factors Associated with Variation in Insulin Clearance

In 75 patients, receiving 62 portal and 68 peripheral infusions of insulin, we examined how clearance of exogenous insulin varies a) with the route of infusion, b) between normo- and hyperglycemia, c) from morning to afternoon, and d) with common indices of metabolism, such as plasma insulin, insulin sensitivity, glucose tolerance and relative body weight (IV). In 45 patients the clearance rate, as well as the secretion rate, of pancreatically released insulin was estimated during steady-state normo- and hyperglycemia (V). In 29 of the latter patients, the clearance of portally infused insulin was also determined.

After intraportal infusion of insulin, $\overline{PCR}^V$ was lower at hyper- than at normoglycemia, the decrease being about 30 %

after the 10 mU/kg dose. It was also slightly lower the higher the relative body weight or the older the patient, but these two correlations were weak. None of the above findings was found after peripheral infusion of insulin. Regardless of the route of insulin infusion, PCR did not vary with the pretest endogenous insulin concentration, the time of the day, the insulin sensitivity or the glucose tolerance. The effect of insulin on blood glucose was smaller in the afternoon than in the morning, whereas the opposite was true for the effect on plasma FFA. The blood glucose decrement at hyperglycemia was similar to that at normoglycemia.

In the basal fasting state, interindividual variation in endogenous arterial insulin levels was related to variation in the clearance rate as well as the secretion rate. During the glucose infusions the height of the endogenous arterial insulin level correlated predominantly with the rate of insulin secretion and not significantly with PCR_{ss} . However, when the relationship between PCR_{ss} and I_a^{ss} was tested separately for the two dose levels of glucose infusion there was a negative correlation between PCR_{ss} and I_a^{ss} ($n = 12$, pooled $r = 0.63$, $P = 0.05$), while the positive correlation between SR_{ss} and I_a^{ss} remained significant (pooled $r = 0.66$, $P < 0.05$) (data not shown in paper V). PCR_{ss} was lower in the basal fasting state (about $11 \text{ ml min}^{-1} \text{ kg}^{-1}$) than during glucose infusion (about $17 \text{ ml min}^{-1} \text{ kg}^{-1}$). PCR_{ss} was lower than PCR (and PCR) at normoglycemia, whereas the two clearance rates were similar during glucose infusion. PCR_{ss} varied significantly between individuals, but there was no correlation between PCR_{ss} and PCR. It did not vary with relative body weight, whereas SR_{ss} did. Neither PCR_{ss} nor SR_{ss} varied with age, insulin sensitivity or glucose tolerance (data not shown in paper V). Fasting plasma insulin and SR_{ss} were lower in the afternoon than in the morning whereas PCR_{ss} did not change.

The results corroborate the previous finding (II) that an acute increase in glucose (and plasma insulin) reduces the fractional uptake of insulin in the liver in the short-term perspective. They also indicate that the effect of glucose on PCR is solely, or predominantly, an effect on the hepatic uptake of insulin. After peripheral insulin infusion, the slower fractional hepatic uptake at hyperglycemia was evident only when the effects of hyperglycemia and dose were combined. Since no difference in PCR was found when the insulin dose was small, it is not possible to exclude a slight increase in nonhepatic clearance at hyperglycemia. The decrease in PCR at hyperglycemia was accompanied by a more convincing decrease in APV (or ADV), corroborating a decrease in FH.

The observation that the clearance rate of immunoreactive insulin is lower in the basal state than otherwise agrees

with the finding of Harding et al. that a continuous infusion of insulin is accompanied by an increase in the clearance of (systemic) insulin (55). One explanation for the difference in PCR_{ss} and PCR values at normoglycemia is that the plasma disappearance curve contains a very slow and small final component that escapes detection. It was recently reported that some of the iodinated insulin, which first seems irreversibly lost, starts to return to plasma some 40 min after the administration of the tracer (135). This late reentry is consistent with reversibility of the binding of insulin (36, 41). However, the quantitative influence of reversible insulin binding appears small as judged from direct studies in vivo (156, 157) and the similar disappearance curves after instant injection and the stopping of an infusion (22). Though other properties of the insulin removal system may be responsible, we believe that poor specificity of the insulin immunoassay at low insulin concentrations plays a dominant role. Proinsulin does not account for the large discrepancy between PCR_{ss} and PCR in the fasting state, nor does it explain the different PCR_{ss} values in the two steady states (normo- and hyperglycemia) (155). Accordingly, part of the insulin immunoreactivity should be due to other, unspecified substances (158), possibly high-molecular-weight complexes of insulin (159, 160), modified insulins (161) and/or degraded insulins (162). The small fractional uptake of insulin in the ESB when endogenous insulin concentrations are low (see above) should not result in any significant underestimation of PCR_{ss} or SR_{ss} .

Gavin et al. (38) demonstrated that the number of insulin receptors decreases as the ambient insulin concentration increases and postulated that this negative feed-back regulation is of physiologic importance. Though challenged (48, 49), the relevance of this mechanism has recently been supported (35). The concept is popular because it readily explains the frequent demonstration of a negative correlation between insulin binding and the fasting plasma insulin concentration (36, 39, 41, 45, 46). Self-regulation has been demonstrated also for other peptide hormones (41). The correlations between PCR_{ss} and I_a^{ss} are consistent with regulation of insulin receptors by insulin itself. But the absence of correlation between pretest endogenous insulin levels and PCR as well as between PCR_{ss} and PCR indicates that the insulin receptor plays a relatively minor role for the disappearance rate of acutely elevated insulin. In agreement herewith, another study (46) shows lack of correlation between the clearance of exogenous insulin and intra-individual changes in fasting plasma insulin, or insulin binding. The explanation must be that after acute elevation of plasma insulin other factors are more important and conceal the coupling between PCR and insulin binding, as represented by I_a^{ss} or PCR_{ss} values. Such factors are the rate

and distribution of blood flow, capillary permeability, rates of diffusion, and compartment volumes. It should be noted that PCR_{ss} was unrelated to PCR also during glucose infusion. Thus, a possibly reduced specificity of the assay at low insulin concentrations can be only partly responsible for the absence of relationships between PCR and PCR_{ss} , or I_a^{ss} .

Interindividual variation of PCR and PCR_{ss} was not related to variation of insulin sensitivity or glucose tolerance. Uncoupling between insulin clearance and insulin action was evident also when comparing the results obtained a) at normo- and hyperglycemia, b) in the morning and in the afternoon, and c) before and after inguinal herniorrhaphy. Disappearance and effect of peripherally administered insulin have been found to be associated (26) and unassociated (12) in normals and in untreated diabetics and to be easily dissociated in obese patients (46). In untreated diabetics, resistance to insulin is accompanied by a normal (12, 18) or a decreased (18, 26) insulin disappearance rate. All these findings imply that the clearance rate is relatively unimportant for the acute effect of insulin on glucose and FFA metabolism. As far as PCR is concerned, the absence of relationship may be partly explained by the lack of correlation between the non steady-state clearance rate and the fasting insulin level, and presumably the receptor binding (see above). Furthermore, insulin action is subject to a variety of hormonal and metabolic factors capable of modulating the post-receptor fate and effect of insulin. There are many situations in which insulin sensitivity, or glucose tolerance, and insulin binding change in opposite directions and/or where relationships between them are absent (36, 37, 39, 40, 45, 46). Tolerance to glucose depends also on the rate of insulin release.

The correlations with relative body weight indicate that increased fasting plasma insulin levels in obese patients are caused mainly by an increased pancreatic secretion rate. An increased secretion is probably also the major cause of the high insulin levels in obese patients after pancreatic stimulation (5, 33, 163), though our results indicate that a somewhat reduced clearance rate may contribute in this situation. The reason why PCR and PCR_{ss} showed different relations to relative weight may be that the factors determining the clearance rates have a varying degree of influence in the non steady- and the steady-states, as discussed above. Available data indicate that plasma and liver volumes and portal blood flow are roughly proportional to body weight within the weight range studied (90, 91, 131, 164, 165). But the reservation must be made that small deviations from these relationships can be concealed by the measurement errors (90, 131). The correlations with PCR_{ss}

and SR_{SS} are valid whether or not PPF is proportional to body weight, whereas the interpretations differ. If PPF did not vary with body weight, the absence of correlation between relative body weight and PCR_{SS} means that the total clearance rate of insulin (in ml/min) was unrelated to the degree of obesity. If plasma volume was not proportional to body weight, the negative correlation between PCR and relative weight is apt to be overestimated (IV). This possible bias is obviated by calculating the total insulin clearance (in ml/min). This measurement was unrelated to relative body weight (pooled partial $r = 0.08$, $P > 0.05$) and is only suggestive of a lower clearance rate per kg body weight in obese patients. In addition, the lack of correlation between FH and relative weight (II) suggests that the influence of obesity on PCR is only small.

It is conceivable that the clearance of insulin varies negatively with age, but available data suggest that this variation is slight (13, 15). The weak correlation between age and clearance rate in our study was not due to a possible covariation with relative body weight (166). It was, however, observed only after portal infusion at normoglycemia and may thus be attributed to random variation (mass significance).

Disappearance of Insulin as a Determinant of Concentration and Action of Plasma Insulin

The clearance of intraportally administered insulin is decreased shortly after increases in glucose and insulin. This is caused by a lowered clearance in the liver and is thus accompanied by a redistribution of insulin from the liver to the periphery (II, IV). The values found for endogenous insulin agree with a reduced hepatic clearance after acute elevation of glucose (II), and demonstrate that variation in clearance plays a role for variation of the plasma insulin level, both at fasting and during hyperglycemia (II, V). However, the insulin secretion rate is probably the most important determinant of the insulin level in plasma. This is indicated by larger changes in the insulin secretion than in the (hepatic) clearance rate after glucose loads (I-V, 1-7). It is also consistent with the finding that the insulin level during glucose infusion correlates better with the secretion rate than with the clearance rate when the two dose levels of glucose infusion are analysed together (V). In addition, when fasting is prolonged to the afternoon, with a consequent lowering of plasma insulin, the secretion rate is lower whereas the clearance rate is not (IV, V). Yet, the sensitivity of the liver to changes in blood glucose infers that the insulin secretion rate is poorly reflected in the concentrations of plasma insulin when blood glucose varies.

It should be observed that only the effects of glucose and insulin have been considered in this study. Though glucose is the most powerful stimulus of insulin secretion, there are also a number of other secretagogues (1-7). Such a number of determinants have not been demonstrated for insulin clearance, but it is likely that this process is controlled by a variety of factors, too (V, 40, 42, 45). The lack of relationships between common clinical or laboratory indices of metabolism and insulin clearance does not contradict this idea.

Insulin action seems to depend primarily on factors that do not affect the degree of insulin clearance. This should not be confused with the finding that the effect of insulin was proportional to the amount of insulin removed, as demonstrated for the body as a whole (blood glucose and FFA decrements) (IV), the extrahepatic splanchnic bed (net glucose uptake) (III), and the liver (net glucose balance) (143). After intraportal insulin, the blood glucose decrement at hyperglycemia was similar to that at normoglycemia despite a smaller hepatic uptake of insulin and a decreased plasma clearance rate. The reason is that the extra insulin diverted to the periphery at hyperglycemia had an effect on nonhepatic tissues approaching that on the liver at normoglycemia. Thus, variation in clearance simultaneously with variation in sites of action is another reason why it may be difficult to demonstrate coupling between clearance rate and effect in the body as a whole.

Considering the body as a whole, the ease with which glucose-induced variation in insulin secretion (1-7) and (hepatic) insulin clearance (II, IV, V) can be demonstrated contrasts with the difficulty to demonstrate variation in the insulin effect on blood glucose (IV, 143). As recently postulated (167), this suggests that the strict regulation of blood glucose is of secondary importance to the maintenance of optimal plasma insulin levels and that one fundamental role of glucose is to assist in attaining adequate insulin levels. In addition, it indicates that one or more of the other effects of insulin (65, 167) are as important, or more important, than that on glucose metabolism. The preference to regulate plasma insulin by blood glucose, rather than the reverse, is compatible with the metabolic changes in the fed and fasted state (65) and the occurrence of spontaneous hypoglycemia in early diabetes (168).

SUMMARY

Disappearance of insulin from plasma was analysed in 90 unanaesthetized patients, 73 of whom had a transumbilical portal catheter. None had diabetes or liver disease, and none was markedly obese. Brief infusions of insulin were given into the portal or a peripheral vein, at normoglycemia and during constant intraportal glucose infusion. Clearance rate of pancreatically released insulin was estimated during steady-state normo- and hyperglycemia.

After infusion of insulin, plasma clearance rate ($\text{ml min}^{-1} \text{kg}^{-1}$) was calculated as dose over the area under the plasma curve. Conventional noncompartmental and compartmental models were of limited value mainly because of the imprecision of the experimental data and the difficulty to define a proper baseline. After intraportal, but not after peripheral, infusion of insulin, the plasma clearance rate was lower the larger the insulin dose and lower at moderate hyperglycemia than at normoglycemia. The decrease in clearance rate was due mainly to a diminished first pass fractional hepatic uptake of insulin in both cases. A rise in glucose resulted in a clear-cut decrease of hepatic insulin clearance at all insulin levels, whereas a rise in insulin alone did so only at insulin concentrations exceeding the physiologic range. A small fraction of insulin was taken up in the extrahepatic splanchnic bed. In the steady-state, interindividual differences in the height of the endogenous insulin concentration were related to differences in the rate of clearance. The clearance of the infused insulin showed no relationship to endogenous levels. The clearance rate of endogenous insulin in the basal unperturbed state was markedly lower than in any other situation. Clearance rates of exogenous or endogenous insulin did not vary with age, the time of the day, glucose tolerance or insulin sensitivity; possibly, clearance of exogenous insulin varied negatively with relative body weight.

In conclusion, it was shown that variation in the clearance rate of insulin does influence the plasma insulin level. It was also shown that an acute increase in glucose is accompanied by a decrease in the hepatic clearance rate of insulin.

APPENDIX^{*}

For derivation of k_{el} in the two-compartment model and the relationships between mean transit time and compartmental analysis it is convenient to derive first the impulse response and the transfer function of the system. Following an impulse or infusion input signal to a linear and stationary system, the mean transit time, $\bar{t}$, is given by (83)

$$\bar{t} = \int_0^{\infty} t \cdot h(t) dt / \int_0^{\infty} h(t) dt \quad (12)$$

where $h(t)$ is the "unit impulse response". The output function, $y(t)$, and the output concentration, $I_1(t)$, are thus given by

$$y(t) = \int_0^t h(s) \cdot m(t-s) ds \quad (13)$$

and

$$y(t) = PCR \cdot I_1(t) \quad (14)$$

where $m(t)$ denotes the infusion rate (input function), and $y(t)$ and $m(t)$ have the dimension amount per time unit.

Denoting the Laplace transforms of y , h , and u by $Y(s)$, $G(s)$, and $M(s)$, equation 13 is written as

$$Y(s) = G(s) \cdot M(s) \quad (15)$$

and equation 12 as

$$\bar{t} = - \frac{1}{G(0)} \left[\frac{dG(s)}{ds} \right]_{s=0} \quad (16)$$

The total dose, D , can be expressed as $D = \int_0^{\infty} m(t) dt = \int_0^{\infty} y(t) dt$ or $D = M(0) = Y(0)$. It follows that $G(0) = 1$.

Calculation of k_{el} . In the two-compartment model (Fig. 1, equation 5), k_{el} can be derived as follows. The net clearance rate from V_1 is denoted PCR and the corresponding net fractional transfer rate constant is called k_{el} (see Calculations). By definition:

$$k_{el} = PCR/V_1 \quad (17)$$

From equation 5, 14, and 15 it follows that

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$$G(s) = \frac{(s+k_{12}+k_{32}) \text{ PCR}/V_1}{s^2 + (k_{12}+k_{21}+k_{32})s + k_{21} k_{32}} \quad (18)$$

$G(0) = 1$ in equation 18 and combination with equation 17 gives

$$k_{el} = k_{21} \cdot k_{32} / (k_{12} + k_{32}) \quad (19)$$

Relationship between mean transit time and compartmental analysis.

In the two-compartment model, combination of equations 16, 18, and 19 gives

$$\bar{t} = - \frac{k_{21} \cdot k_{32}}{(k_{12}+k_{32})} \cdot \frac{k_{21} \cdot k_{32} - (k_{12}+k_{32}) (k_{12}+k_{21}+k_{32})}{(k_{21} \cdot k_{32})^2} =$$

$$\frac{1}{k_{el}} + \frac{k_{12}}{k_{32} (k_{12}+k_{32})} \quad (20)$$

Combination of equations 3, 17, and 20 also gives

$$\text{ADV} = V_1 \left[1 + \frac{k_{12} \cdot k_{21}}{(k_{12} + k_{32})^2} \right] \quad (21)$$

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Hepatic uptake of insulin in man

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ABSTRACT

The hepatic uptake of unlabeled insulin (5-50 mU/kg) was determined in 45 nondiabetic patients without hepatic disease. Thirty patients were studied at normoglycemia and 15 at moderate steady hyperglycemia, induced by intraportal infusion of 80 or 200 mg glucose per h and kg. The first pass fractional hepatic uptake of insulin (FH) was estimated by comparing the results after a portal and a peripheral infusion of unlabeled insulin in each patient. It varied negatively with the amount of insulin given, decreasing about 50 % after a tenfold increase in dose. FH was markedly smaller during steady hyperglycemia than at normoglycemia, regardless of insulin dose. For low doses (5-10 mU/kg), giving insulin concentrations within the physiologic range, FH was 0.43 (5 mU/kg) and 0.40 (10 mU/kg) at normoglycemia and 0.16 (10 mU/kg) at hyperglycemia. FH did not vary with the fasting, or the glucose-stimulated, endogenous insulin levels recorded before insulin infusion. It is suggested that glucose is more important than insulin in modulating the fractional hepatic uptake of insulin.

plasma clearance rate; dose-dependence; glucose infusion;
liver; portal vein

All insulin secreted into the blood enters the portal vein and passes the liver. As a consequence, variation in the fractional hepatic uptake may markedly affect the distribution and removal of secreted insulin and cause considerable variation of peripheral insulin levels not accounted for by variation in secretion rate. It should also have important consequences for the homeostasis of glucose and other nutrients (7). The liver is known to remove a substantial amount of the insulin reaching it, but it is unsettled whether the fractional hepatic uptake is constant or not. According to studies in vivo, it decreases (11, 12), remains unchanged (15, 30) or increases (8, 9, 21) with increasing portal concentration of insulin. Hyperglycemia, together with hyperinsulinemia, has likewise been shown to be associated with a decrease (22) and an increase (8, 9, 21) in fractional insulin uptake by the liver. These discrepancies might be due to differing experimental conditions. They might also be due to methodological difficulties arising from the anatomic position and the vascular arrangements of the liver.

We measured the first pass hepatic uptake of insulin in unanesthetized, postabsorptive man at normoglycemia and steady hyperglycemia. It was determined after a brief infusion of unlabeled insulin into the portal and a peripheral vein in each subject. Since only peripheral plasma insulin concentrations were compared, we avoided the problems connected with the sampling of portal and hepatic venous blood (15, 31).

MATERIAL AND METHODS

Subjects. a) Hepatic uptake of insulin was studied in 45 patients with a transumbilical portal catheter (Table 1). All of them except 1 were to be, or had been, treated for malignant disease, but none had demonstrable distant metastases. The portal catheter had been inserted under general anesthesia (13), 2 days before portography for possible liver metastases (13), and 3 days before the experimental studies. The day after portal catheterization the patient resumed his preoperative routine, moved about freely in the ward, and had an ordinary hospital diet. Only patients in whom portography, liver scintigraphy and subsequent laparotomy did not reveal liver metastases or porto-systemic shunting were included. No major operation was performed within 6 months before the studies. The results of standard liver and kidney laboratory tests were normal. All had normal fasting blood glucose concentrations. None had prior evidence of endocrine disease and none was receiving drugs known to disturb insulin or carbohydrate metabolism.

b) In a subgroup consisting of 10 of the above and 15 other patients the portal and arterial insulin concentrations were compared following an intravenous pulse of glucose. The 15 patients resembled the main group so closely that it was not

TABLE 1. Clinical data

No. of subjects	Age, yr	Sex, M/F	Body wt, kg	Relative wt* wt*	Diagnosis		Fasting		$k_G^{\dagger}$, %/min
					Neoplasms [†]	Sigmoiditis	Blood Glucose, mmol/l	Plasma Insulin, μ U/ml	
45	63.8 $\pm$ 7.7 44-82	27/18	69.4 $\pm$ 12.3 50-95	1.10 $\pm$ 0.12 0.86-1.41	36 (8)	1	4.36 $\pm$ 0.49	12.0(5.4-16.0)	1.00 $\pm$ 0.28
							3.22-5.33	1-26	0.42-1.68

First line gives means $\pm$ SD, and for plasma insulin median and 20th and 80th percentiles. Second line gives the ranges. * Tables of Metropolitan Life Insurance Company. [†] Carcinoma of the colon or, shown within parenthesis, of the urinary tract. $k_G^{\dagger}$ Rate constant for glucose disappearance during the intravenous glucose tolerance test (IVGTT) (25).

during 2 min. Samples were then taken at 62, 64, 66, 69, 72, 76, 80, 85, 90, 110 and 120 min. In group b (25 patients, including 10 of the above), also portal blood was sampled, 10 s after the arterial blood.

Analytical methods. Blood glucose was determined with a glucose oxidase method (17). Plasma was separated rapidly and stored at -20°C until determination of total insulin immuno-reactivity (16). The insulin antiserum reacts with proinsulin and does not distinguish between the endogenous human and the exogenous porcine insulin. Details of our assay for insulin have been given elsewhere (37).

Calculations. It was assumed that the endogenous arterial insulin concentration remained constant throughout the clearance of exogenous insulin. After infusion of insulin, calculations were thus performed on insulin levels above the pretest baseline.

After portal insulin infusion, the plasma clearance rate, $\text{PCR} (\text{ml min}^{-1} \text{ kg}^{-1})^{\text{v}}$, was defined as (37)

$$\text{PCR}^{\text{v}} = \text{D}^{\text{v}} / \int_0^{\infty} \text{I}_1^{\text{v}}(t) dt \quad (1)$$

$\text{D} (\mu\text{U/kg})$ denotes the dose and $\text{I}_1(t) (\mu\text{U/ml})$ the concentration of plasma insulin in arterial blood. With this definition, it follows that after portal infusion the clearance rate corresponding to the first pass fractional hepatic uptake of insulin, FH, is included in PCR (Fig. 1).

After peripheral infusion of insulin, PCR was similarly defined as

$$\text{PCR}^{\wedge} = \hat{\text{D}} / \int_0^{\infty} \hat{\text{I}}_1(t) dt \quad (2)$$

After portal infusion, the posthepatic dose is designated $\text{D}^* (\mu\text{U/kg})$ and represents molecules escaping the first pass hepatic uptake. By definition:

$$\text{D}^* = (1 - \text{FH}) \text{D}^{\text{v}} \quad (3)$$

Rearrangement gives

$$\text{FH} = 1 - \text{D}^* / \text{D}^{\text{v}} \quad (4)$$

The first pass total hepatic uptake of insulin, FT ($\mu\text{U/kg}$),

¹ The superscripts v and $\wedge$ denote data from respectively the portal and the peripheral routes of infusion. The term post-hepatic denotes insulin escaping the first pass hepatic uptake after portal infusion.

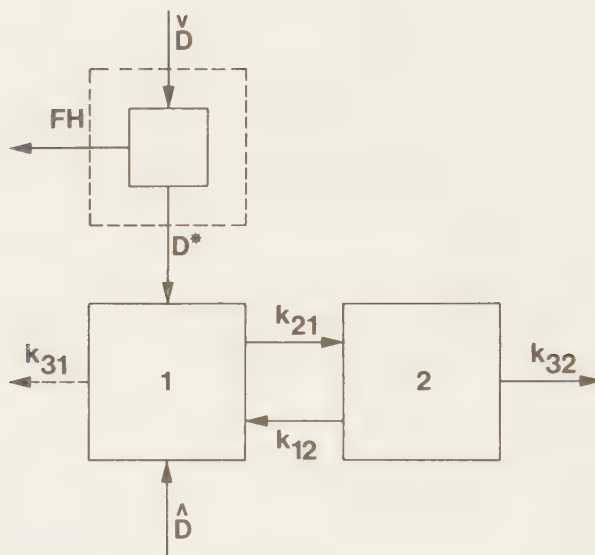


FIG. 1. Compartmental model for portal and peripheral infusion of insulin ($\bar{D}$, portal dose; $\hat{D}$, peripheral dose). After portal infusion, the first pass fractional hepatic uptake is designated FH. The first pass hepatic uptake is treated as an instantaneous loss meaning that $(1 - FH)\bar{D} = D^*$ (post-hepatic dose) reaches the systemic circulation. The hepatic removal of posthepatic (recirculating), as well as of peripherally administered, insulin is lumped together with the removal in other tissues. For molecules entering the systemic circulation, flow between intravascular (1) and extravascular (2) compartments has rate constants k_{21} and k_{12} and the irreversible loss has rate constant k_{32} (or k_{31}).

is given by $FT = \frac{V}{D} - D^* = FH \cdot \frac{V}{D}$

The first pass hepatic uptake can thus be estimated by determining the amount of exogenous insulin that reaches the systemic circulation after infusion into the portal vein. A natural approach is to assume that the clearance rate for posthepatic insulin after intraportal infusion, i.e., for D^* , is identical with that for peripherally infused insulin (26). The following mass balance equation is then obtained

$$D^* = PCR \int_0^{\infty} \frac{V}{I_1}(t) dt \quad (5)$$

Combination with equations 1 and 4 gives that

$$FH = 1 - PCR / PCR \quad (6)$$

Pilo et al. derived equation 6 in a somewhat different way (26). It involves no assumptions about the compartmental structure.

Instead we chose an approach that does not require the above assumption of identical clearance rates for insulin reaching systemic blood after the two infusions. We used compartmental analysis to estimate k_{el} (min^{-1}), the average net fractional transfer rate constant from plasma to the lumped extravascular compartment. All portal and peripheral infusions were fitted to a monocompartment model, and a two-compartment model with irreversible loss from the extravascular compartment (Fig. 1). The model with the "best fit" was then chosen. The analysis comprised only those insulin molecules that entered the systemic circulation. The first pass hepatic uptake was thus treated as an instantaneous loss. The hepatic uptake of posthepatic (recirculating), as well as that of peripherally infused, molecules was lumped with the uptake in other tissues. The equations were:

$$\begin{aligned} V_1 \cdot dI_1(t)/dt &= -V_1 \cdot k_{21} \cdot I_1(t) + V_2 \cdot k_{12} \cdot I_2(t) + V_1 \cdot B_0 \cdot U(t) \\ V_2 \cdot dI_2(t)/dt &= -V_2 \cdot (k_{12} + k_{32}) \cdot I_2(t) + V_1 \cdot k_{21} \cdot I_1(t) \\ U(t) &= 1 \text{ for } 0 \leq t \leq 2.5 \\ &= 0 \text{ for } 2.5 < t \end{aligned} \quad (7)$$

The k_{ij} 's are rate constants, $I_1(t)$ and $I_2(t)$ are the insulin concentrations in plasma and extravascular compartment(s), respectively, V_1 and V_2 the corresponding volumes and $B_0 \cdot U(t)$ the computer-fitted infusion rate in $\mu\text{U}/\text{min}$ per ml of V_1 from 0 to 2.5 min. In the chosen two-compartment model, k_{el} is equal to $k_{21} \cdot k_{32} / (k_{12} + k_{32})$. If it is assumed that

irreversible exit is from the plasma pool (see Fig. 1), k_{el} ($=k_{31}$) will be identically large. Fitting to experimental data points was started at 4 min, at which time intravascular mixing was assumed complete. Details of the computational procedures and the criteria used to select the mono- or two-compartment model have been presented elsewhere (37). Briefly, the two-compartment model was chosen when it had the lower AIC (Akaike's information criterion) value (1) and its transfer rate constants and V_1 values were not meaningless or unreasonable (e.g. negative).

The apparent plasma volume, $\hat{APV}$ ($=V_1$) (ml/kg), for insulin entering the systemic circulation can be defined and calculated as (37)

$$\hat{APV} = \hat{PCR} / \hat{k}_{el} \quad (8)$$

Assuming that this volume is the same during the portal experiment, the following mass balance equation is obtained for posthepatic insulin

$$D^* = \hat{k}_{el} \cdot \hat{APV} \cdot \int_0^{\infty} I_1(t) dt = \hat{k}_{el} \cdot \hat{APV} \cdot (\hat{D}/PCR) \quad (9)$$

The second equality follows from equation 1. In analogy with equation 8, one may introduce the fictive volume $\hat{APV}$ by

$$\hat{APV} = \hat{PCR} / \hat{k}_{el} \quad (10)$$

and write equation 9 as

$$D^* = \hat{D} \cdot \hat{APV} / \hat{APV} \quad (11)$$

Combination of equations 4 and 11 gives

$$FH = 1 - \hat{APV} / \hat{APV} \quad (12)$$

If $\hat{k}_{el}$ were equal to $\hat{k}_{el}$, equation 6 would be the same as equation 12. Unless otherwise stated, equation 12 was used in calculating FH.

The mean transit time, $\bar{t}$ (min), was calculated as

$$\bar{t} = \left(\int_0^{\infty} t \cdot I_1(t) dt / \int_0^{\infty} I_1(t) dt \right) - T/2 \quad (13)$$

where correction is made for the $T = 2.5$ min insulin infusion (37). Assuming that posthepatic and peripherally introduced insulin have identical mean transit times, the hepatic mean transit time, $\bar{t}_h$ (min), may be calculated as (26)

$$\bar{t}_h = (\bar{t}) - (\bar{t}) \quad (14)$$

The underlying assumption is analogous to that made in the

derivation of equation 6. Equation 14 was introduced mainly to further elucidate the disappearance of posthepatic insulin.

The areas in equation 1, 2 and 13 were calculated by trapezoidal integration and exponential extrapolation of the tail of the curve. The loss of insulin due to adsorption to syringes and catheters was 14 - 16 % (37). It was corrected for in all calculations, but the uncorrected dose is given in the text.

The arterial blood glucose decrement, BGD (%), after infusion of insulin was calculated as the percentual change from the pretest level to the mean of the two lowest consecutive concentrations. The k_g values of IVGTT were determined as proposed by O'Sullivan et al. (25) and are given in Table 1.

Statistical methods. Standard statistical methods were used (32). Log transformation of fasting plasma insulin normalized the distribution and was thus performed before statistical analysis. Since logarithmic transformation of D gave a good overall linear correlation with FH, such transformation was also performed before statistical analysis. Unless otherwise indicated, values given are means $\pm$ SD.

RESULTS

Table 2 gives the values of FH, PCR, k_{el} , APV, and BGD for each individual study. Sets of primary data are given in Figs. 2-3. For 9 infusions the plasma insulin disappearance curve was described by two exponentials. Otherwise a single exponential was used (Table 2).

The PCR values are summarized in Fig. 4. PCR did not vary with the glucose infusion rate (80 or 200 mg h^{-1} kg^{-1}) and the results at hyperglycemia were therefore pooled. After portal infusion of insulin, PCR was clearly dose-dependent at normoglycemia ($r = -0.72$, $P < 0.001$), but not at hyperglycemia ($r = -0.48$, $P < 0.10$). PCR values were not significantly smaller at hyper- than at normoglycemia (analysis of covariance, $P < 0.10$). It should be noted that the dose-dependence of PCR required comparison of regression lines, which, however, may not be an ideal method of comparison (Fig. 4). The absence of difference in PCR between normo- and hyperglycemia should therefore be interpreted with caution. After peripheral infusion of insulin, PCR varied negatively with dose at hyperglycemia ($r = -0.56$, $P < 0.05$), but not at normoglycemia ($r = -0.14$, $P > 0.05$).

Hepatic Uptake of Insulin

The values for first pass hepatic uptake of insulin are

TABLE 2. Kinetic parameters for each infusion

Glucose Infusion													
Rate, mg h ⁻¹ kg ⁻¹		Portal Insulin Infusion						Peripheral Insulin Infusion					
Patient no.	Pretest BG, mmol l ⁻¹	Pretest I ₁ , μU ml ⁻¹	D, mU kg ⁻¹	PCR, ml min ⁻¹ kg ⁻¹	k _{el} [*] min ⁻¹	APV, ml kg ⁻¹	BGD, %	D, mU kg ⁻¹	PCR, ml min ⁻¹ kg ⁻¹	k _{el} [*] min ⁻¹	APV, ml kg ⁻¹	BGD, %	FH
0													
1	4.72	4	5	47.39	0.513	92.37		5	16.33	0.515	31.71		0.657
2	4.44	8	5	41.31	0.389	106.20	9.3		21.78	0.355	61.35	10.3	0.422
3	5.00	4	5	28.03	0.349	80.32		5	20.39	0.395	51.62		0.357
4	4.94	1	5	21.43	0.415	51.63	8.0	5	16.89	0.419	40.30	7.0	0.219
5	5.17	26	5	22.23	0.342	64.99	7.5	5	8.04	0.236	34.07	8.6	0.476
6	4.33	21	10	37.01	0.361	102.52	10.0	10	20.60	0.325	63.46	7.8	0.381
7	4.39	8	10	37.11	0.260	142.73	11.4	5	11.80	0.232	50.78	11.7	0.644
8*	3.56	6	10	19.38	0.265	73.11	10.9	5	10.68	0.260	41.08	8.6	0.438
9	3.94	8	10	32.61	0.354	92.12	14.1	10	12.24	0.286	42.79	4.15	0.415
10	4.61	18	10	21.36	0.266	80.31	8.4	5	20.64	0.361	57.18	4.1	0.379
11	4.61	4	10	26.21	0.350	74.89	9.6	5	12.64	0.278	45.51	7.3	0.433
12	5.33	12	10	21.00	0.329	63.82	7.3	5	15.32	0.404	52.78	8.6	0.295
13†	4.28	9	10	29.61	0.303	97.71	12.3	5	18.88	0.416	38.18	10.8	0.402
14	4.06	9	10	31.84	0.414	82.65		5	18.22	0.328	55.54	4.2	0.432
3	5.33	6	10	33.83	0.474	71.38		5	20.39	0.395	51.62		0.328
14	4.78	10	20	22.37	0.300	74.58	25.4	20	18.73	0.402*	46.59	20.7	0.375
15†	4.78	16	20	16.49	0.279	59.11	16.3	15	15.03	0.308	48.80	12.1	0.174
16	4.44	15	20	26.66	0.397	67.16	15.9						0.273
17	4.67	1	20	16.86	0.336†	50.19	13.3	15	10.96	0.214	51.20	18.1	-0.020\$
18	4.11	5	20	25.32	0.355	71.33		15	16.11	0.337	47.80		0.330
19	4.72	10	20	16.96	0.250	67.83	24.7	15	11.38	0.233	48.84		0.280
19	3.67	14	30	14.25	0.213	66.91	20.8	10	11.95	0.245	48.79	3.5	0.271
20	3.72	14	30	22.03	0.257	85.73	12.9	10	22.68	0.370	61.29	5.5	0.285
21	4.00	4	30	27.38	0.261	104.89	19.9	10	25.36	0.370	68.54	8.1	0.307
22	4.33	12	30	19.76	0.311	63.54	17.9	10	20.42	0.415	49.20	12.5	0.266
23	5.06	23	30	9.61	0.198	48.54	20.9	10	10.69	0.329	32.50	10.3	0.330
24	3.22	15	40	11.48	0.173	66.33	18.1	30	12.84	0.194	66.18	25.4	0.002\$
25	4.50	14	40	18.98	0.235	80.76	23.5	30	20.49	0.301*	68.19	21.2	0.156
26	4.28	22	40	20.07	0.218†	92.07	22.1	30	15.42	0.254*	60.70	21.6	0.341

27	3.72	12	50	13.65	0.186	73.37	35.8	20	12.15	0.256 [†]	47.07	14.9	0.358
28	4.11	14	50	10.01	0.143	69.99	38.7	20	12.96	0.259 [†]	50.05	12.9	0.285
29*	4.17	20	50	15.00	0.148	101.36	34.0	20	15.32	0.182	84.18	15.7	0.169
14	3.33	8	50	17.84	0.324 [†]	55.05	24.1	20	16.04	0.194	82.79	15.7	0.183
30	4.00	20	50	14.08	0.210	67.04	22.2	20	18.73	0.402 [†]	46.59	20.7	0.154
80									12.70	0.227	55.95	23.8	0.165
31	5.89	16	10	17.34	0.373	46.48	20.8	5	21.98	0.672	32.71	5.3	0.296
32	5.47	21	10	19.23	0.340	56.57	16.2	5	14.99	0.289	51.86	5.6	0.083
33	5.44	12	10	27.91	0.369	75.64	6.6	5	16.62	0.265	62.71	7.5	0.171
34	5.22	4	20	16.99	0.317	53.60	15.4	15	19.08	0.270	51.57	8.8	0.038
35	4.78	4	20	25.27	0.417	60.61	18.0	15	16.11	0.337	47.80	14.6	0.271
36	5.78	23	20	19.95	0.310	64.36	13.9	15	11.38	0.233	48.84		0.241
37	4.33	10	40	14.31	0.178	80.38	28.2	20	14.55	0.184	79.08	23.8	0.016
38	4.78	25	40	11.34	0.225	50.41	28.5	30	9.97	0.239	41.72	20.4	0.172
39	7.00	35	10	10.61	0.242	43.83	2.4	5	12.19	0.353	34.52	2.1	0.212
40	5.78	29	10	29.81	0.486	61.35	0	5	17.33	0.297	58.36	0	0.049
41	6.39	23	20	24.54	0.308	79.66	8.2	15	14.52	0.256 [†]	56.72	8.9	0.208
42	8.11	31	20	19.20	0.247 [†]	77.73	8.2	15	15.01	0.190 [†]	78.98	9.8	-0.016
43	7.44	30	20	31.60	0.366	86.43	6.3	20	16.20	0.232	69.85	10.7	0.131
44	7.39	70	40	16.82	0.252	66.73	24.8	30	10.41	0.149	69.84		-0.047
45	6.50	33	40	12.75	0.246	51.83		30	13.86	0.295	46.99	29.2	0.093

8G, arterial blood glucose concentration; I₁, arterial plasma insulin concentration; $\bar{V}$, portal dose of insulin; $\bar{V}_p$, peripheral dose of insulin; PCR, plasma clearance rate; k_{el} , net fractional transfer rate constant from the systemic plasma volume; APV, apparent plasma volume; 8G0, arterial blood glucose decrement; FH, first pass fractional hepatic uptake of insulin. *Peripheral infusion performed twice. †Portal infusion performed twice with the same portal dose. ‡Two-compartment match. § Significantly deviating value (32).

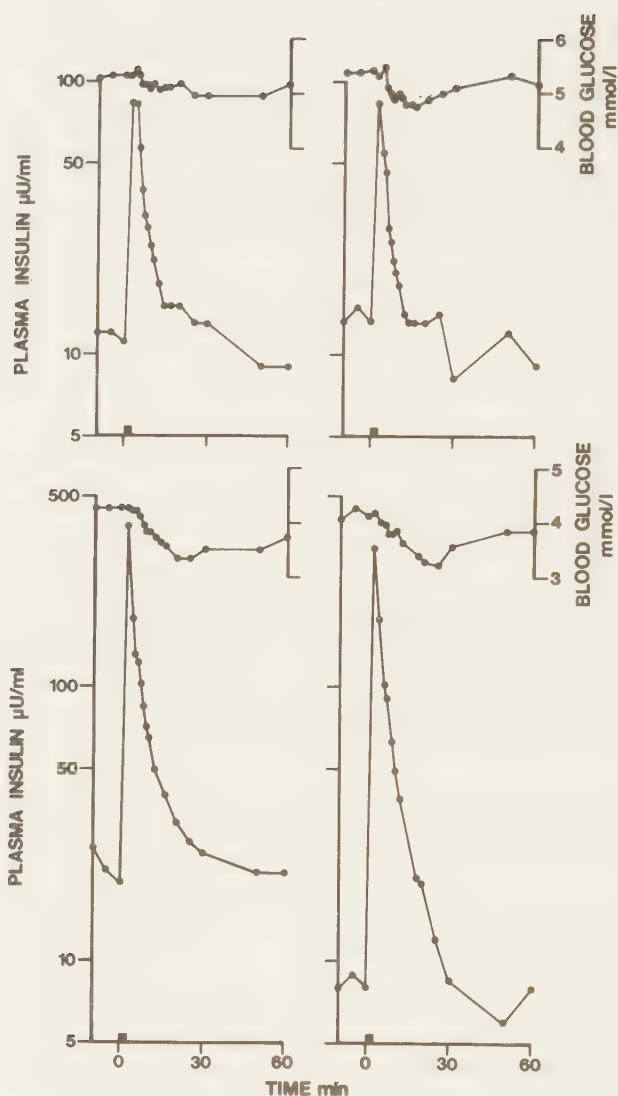


FIG. 2. Arterial plasma insulin and blood glucose concentrations before and after infusion of insulin at normoglycemia. Values after portal infusion (left) and after peripheral infusion (right) in the same patient. Plasma insulin values are logarithmic. Baseline values are not subtracted. Top panel: 10 mU/kg into the portal and 5 mU/kg into a peripheral vein (patient no. 12 in Table 2). Bottom panel: 40 mU/kg into the portal vein and 30 mU/kg into a peripheral vein (patient no. 26 in Table 2).

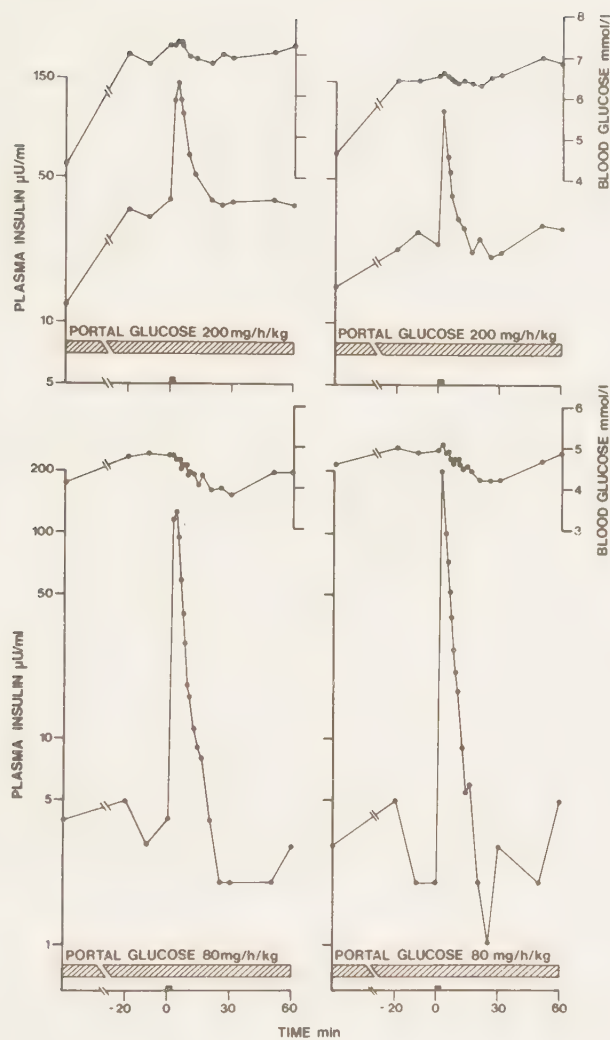


FIG. 3. Arterial plasma insulin and blood glucose concentrations before and after infusion of insulin at hyperglycemia. See legend to Fig. 2. Top panel: 10 mU/kg into the portal vein, 5 mU/kg into a peripheral vein (patient no. 39 in Table 2). Bottom panel: 20 mU/kg into the portal vein, 15 mU/kg into a peripheral vein (patient no. 35 in Table 2).

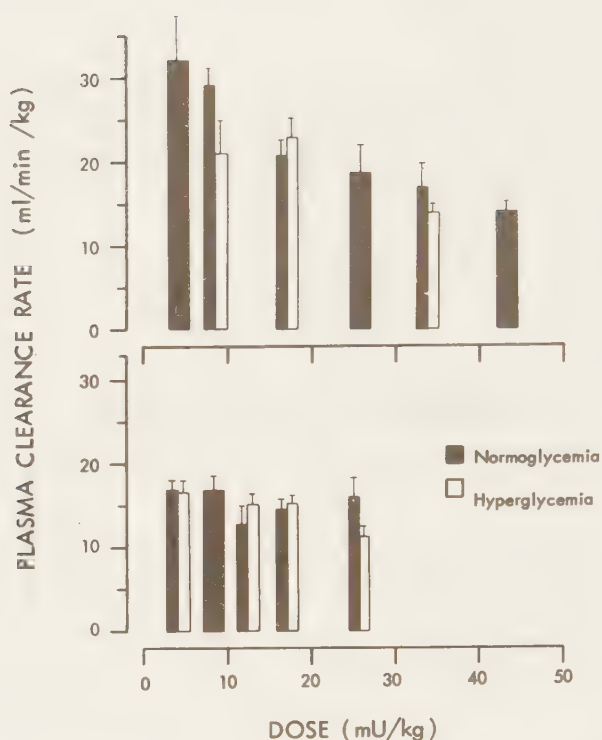


FIG. 4. Plasma clearance rate (PCR) as a function of dose. Top panel: values after portal infusion. Bottom panel: values after peripheral infusion. The results obtained during the two levels of moderate steady hyperglycemia have been grouped. Values are means $\pm$ SEM.

summarized in Fig. 5.² FH varied negatively with insulin dose at normoglycemia ($r = -0.64$, $P < 0.001$), but not at hyperglycemia ($r = -0.37$, $P > 0.05$). It was markedly smaller at steady hyperglycemia than at normoglycemia (analysis of covariance, $P < 0.001$). FH did not vary significantly with the rate of glucose infusion ($P > 0.05$).

Following the portal infusions of 5 and 10 mU/kg at normoglycemia, FH averaged 0.43 ± 0.16 and 0.40 ± 0.10 , respectively ($P > 0.05$). After infusion of 10 mU/kg at hyperglycemia

² As indicated in Table 2, two patients studied at normoglycemia showed significantly deviating results (32) for FH. They were thus excluded from the following analysis as well as from Figs. 5-6.

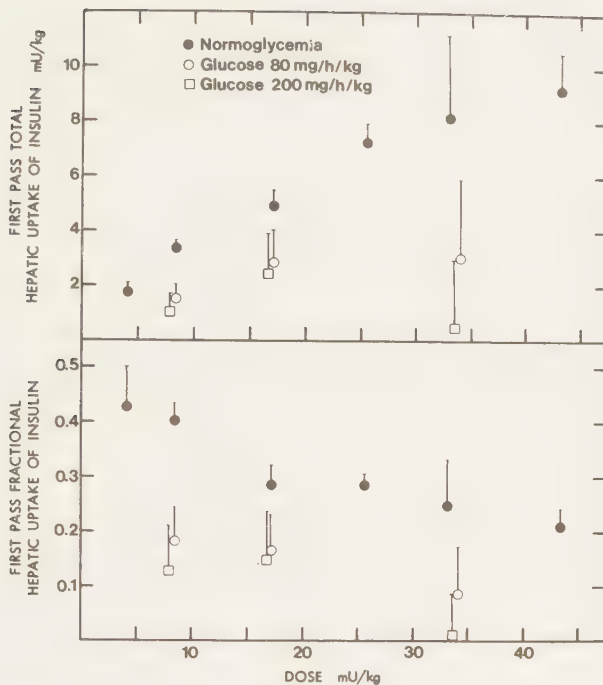


FIG. 5. First pass hepatic uptake of insulin as a function of dose. Values are means $\pm$ SEM.

(80 and 200 mg h⁻¹ kg⁻¹), FH was 0.16 ± 0.10 , which was smaller than at normoglycemia ($P < 0.001$).

At normoglycemia the kinetic properties could be described further, whereas at hyperglycemia the greater variation and the limited number of experiments precluded any further meaningful characterization. Fig. 6 shows a Lineweaver-Burk plot of the inverse FT versus the inverse D. The regression line was calculated according to Barber et al. because $v_{\max}$ was the main source of variance (2). The data were compatible with Michaelis-Menten kinetics, the correlation coefficient being 0.83 ($P < 0.001$). The Michaelis constant, K_m , was 32.0 (23.0-41.0) (mean and 95 % confidence intervals) mU/kg which corresponds to an insulin level of about 1300 (900-1600) μ U/ml in portal vein plasma. The only conclusion that could be drawn from the values at hyperglycemia was that $v_{\max}$ was lower than at normoglycemia. In the Scatchard plot (not shown) the relationship seemed to be curvilinear (concave upwards), but the variation of the values was considerable.

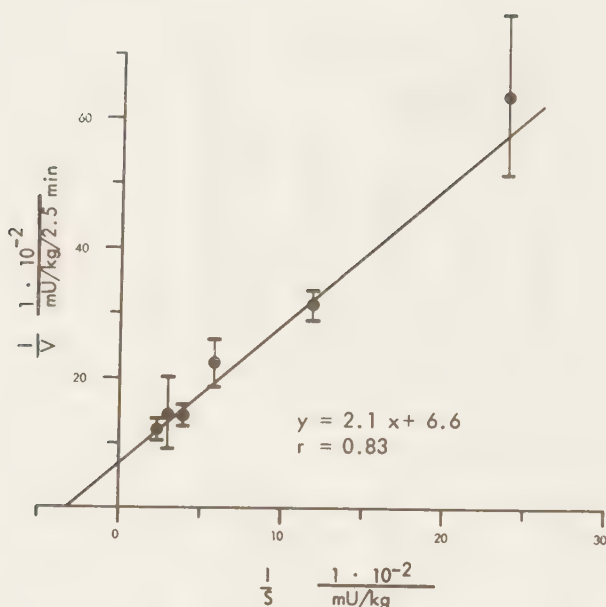


FIG. 6. Lineweaver-Burk plot of the relationship between insulin dose (S) and first pass hepatic removal rate (V) at normoglycemia. The regression line was calculated according to Barber et al. (2). Values are means $\pm$ SEM.

Possible relationships between FH, on one hand, and pretest endogenous blood glucose and plasma insulin levels, relative body weight and k_G , on the other, were studied utilizing the FH versus log D linear relationship (partial correlation analysis). No significant relationship was found, neither at normo- nor at hyperglycemia. At normoglycemia, the partial correlation (r) between FH and fasting blood glucose was -0.29 ($P < 0.10$). Neither did the pretest endogenous insulin levels in the portal vein vary with FH ($n = 9$ and 10 at respectively normo- and hyperglycemia).

As seen in Table 2, two patients had significantly smaller FH values than the other. They had comparatively low k_G values, but another two patients with still smaller k_G values showed normal hepatic removal of insulin.

Despite the assumed linearity, we finally tested whether first pass occupancy of hepatic insulin receptors might influence the clearance rate of posthepatic insulin in the short-term perspective (up to and including 30 min). This was

done by comparing the $(1 - FH)PCR$ values with the corresponding PCR values. When the portal dose was above 20 mU/kg, the $(1 - FH)PCR$ value was significantly smaller than the PCR value in the one and same patient ($P < 0.001$). No such difference could be demonstrated after smaller doses. There was also a negative correlation between D^* and $(1 - FH)PCR$ at normoglycemia ($r = -0.45$, $P < 0.01$), but not at hyperglycemia ($r = -0.30$, $P > 0.05$).

The FH value calculated from equation 6 was less reproducible than the FH value from equation 12. Thus, with equation 6 the coefficient of variation of FH (duplicate determinations with the same portal dose in 4 patients) was 45.6 %; with equation 12 it was 17.4 %. In addition, after portal infusion of more than 20 mU/kg equation 6 gave FH values around or below 0. After smaller doses equation 6 gave mean values close to those obtained with equation 12, though the variances were larger. For the 10 mU/kg portal dose, it gave mean FH values of 0.40 ± 0.13 and 0.13 ± 0.32 at normo- and hyperglycemia, respectively. A similar variation with the dose was observed for the estimated hepatic mean transit time, t_h (equation 14). After doses of 5-10 mU/kg it was 37 ± 43 s, significantly different from 0 ($P < 0.001$). After larger doses it was substantially longer (1-2 min). It was somewhat shorter, though not significantly ($P > 0.05$), at hyper- than at normoglycemia.

Endogenous Portal and Arterial Insulin Levels during the IVGTT

The mean increments in portal and arterial plasma insulin during the IVGTT:s are given in Fig. 7. For comparison, Fig. 7 includes the calculated portal, and the measured arterial, plasma insulin levels after portal infusion of 5 and 10 mU/kg. During the initial part of the IVGTT:s, the endogenous portal-arterial gradient for plasma insulin was considerably smaller than that after exogenous insulin at normoglycemia and also, though less markedly, at moderate steady hyperglycemia.

DISCUSSION

This study showed that the fractional hepatic uptake of insulin decreases with increasing insulin dose and that it is lower during induced hyperglycemia than at fasting.

Calculation of FH based on APV (equation 12), rather than on PCR (equation 6), was preferable because of the better reproducibility of the results. It was also necessary because the data clearly indicated that the clearance rates for peripherally introduced and posthepatic insulin were sometimes markedly different. The greater intraindividual

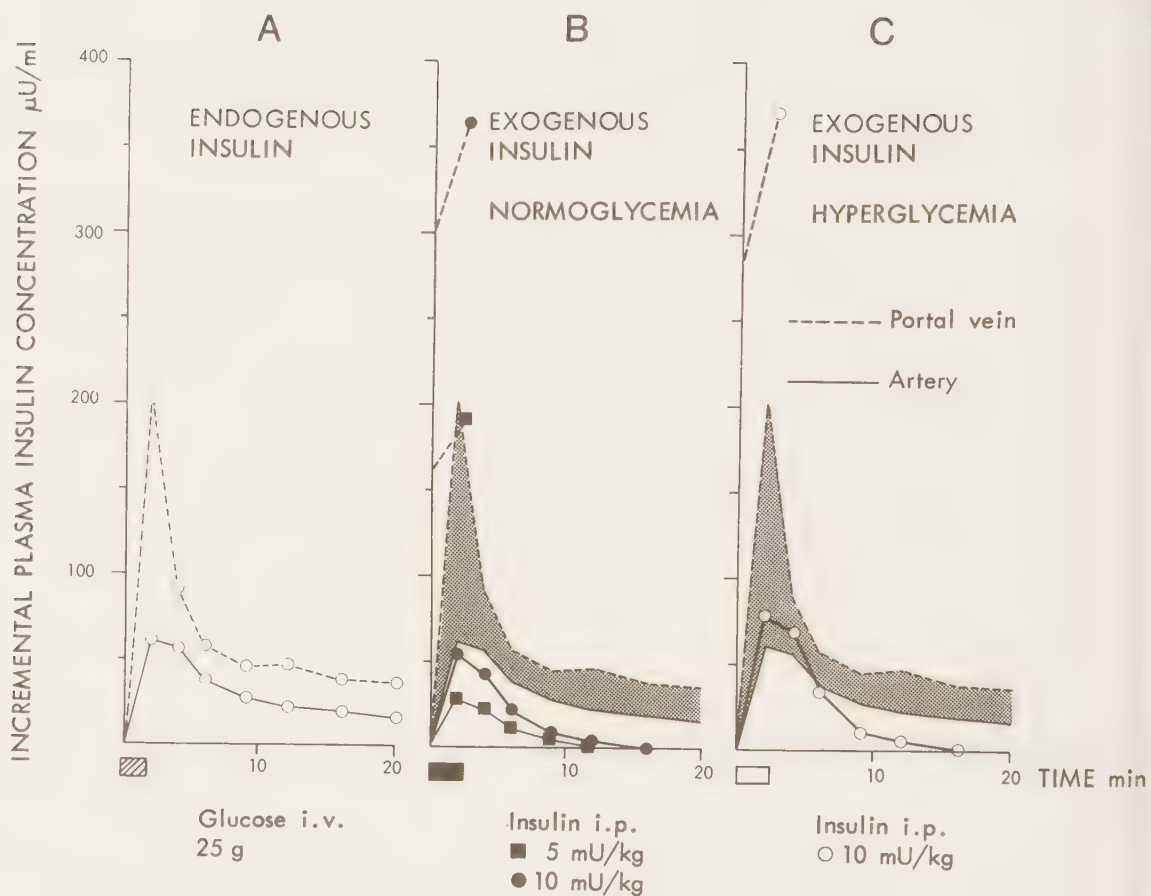


FIG. 7. A: Mean endogenous plasma insulin concentrations in the portal vein and a radial artery following 25 g glucose intravenously (i.v.). B: Mean exogenous arterial plasma insulin concentrations following 5 and 10 mU/kg insulin infusions intraportally (i.p.) at normoglycemia. C: Mean exogenous arterial plasma insulin concentrations following 10 mU/kg insulin infusion i.p. during steady hyperglycemia (80 or 200 mg glucose i.p. per h and kg). Portal vein insulin concentrations during infusion of insulin were not measured, but estimated from the insulin infusion rate and portal plasma flow (14).

reproducibility of FH calculated from APV is consistent with the distinctly smaller intraindividual variation of APV than of PCR or k_{el} (37). With reference to equations 8 and 10, the explanation should be that a large insulin area, giving a small PCR value, is usually accompanied by a relatively small k_{el} value. This covariation between PCR and k_{el} is seen in Table 2. The good reproducibility of APV is consistent with the observation that plasma volume varies but little from day to day within a normal subject (36). When FH is estimated from areas, i.e., equation 6, the variation in clearance rate will affect the estimation of FH because it is assumed that the $(1 - FH)PCR$ value after the portal infusion is equal to the PCR value obtained after the peripheral infusion in the other experiment. In addition, calculation based on PCR gives too small estimates of FH if first pass occupancy of hepatic insulin receptors decreases the clearance rate of posthepatic insulin. This is the plausible explanation for the very small or negative FH values obtained by equation 6 after portal doses exceeding 20 mU/kg. After these doses there was also a large increase in t_h , i.e., in $(\bar{t}) - (t)$, which may be explained by first pass occupancy in the liver, too. It is less likely that the hepatic mean transit time during the first liver passage increases with the dose of insulin. Since unlabeled insulin has been shown to cause a slight displacement of labeled insulin across the hindlimb of the dog (34), one might also imagine that the dose-dependent increase in t_h was due to displacement of endogenous insulin in the liver after large insulin doses with a consequent later appearance of posthepatic insulin. But the observation that the estimated t_h values were not longer at hyper- than at normoglycemia argues against this possibility. In addition, Franckson & Ooms found no hepatic release of insulin after discontinuation of a peripheral infusion of insulin (11), which also indicates that insulin initially removed by the liver was not subsequently released.

The compartmental approach should be regarded only as a mathematical tool to calculate k_{el} and with its help attain a stable reference volume. But otherwise the meaningfulness of the compartmental analysis is questionable (37). The variation (of PCR and FH) with dose and the variation (of FH) with blood glucose mean that the assumptions of linearity and stationarity were inappropriate. It should be observed that this applies also to the calculation of FH from equation 6. Yet, as far as the shortcomings of modeling are concerned, it appears that it is mainly the relative imprecision of the experimental data that is responsible (37).

An important consequence of the limitations of compartmental analysis is that APV varies positively with $\hat{D}$ (37). This correlation is mainly a result of a difference in APV values after the 5-10 mU/kg doses, on one hand, and larger doses,

on the other, the difference being about 20 % of the smaller value (Table 2). As a consequence, it is preferable to match the doses so that the arterial concentrations are similar after the two routes of infusion, as in most of the present studies. Since APV does not vary with the dose for doses exceeding 10 mU/kg (Table 2, ref. 37) it may, however, be adequate to compare APV values after a portal dose of 50 mU/kg and a peripheral dose of 20 mU/kg, as in 5 of the patients (Table 2). In the patients given 30 mU/kg intraportally, the peripheral dose of 10 mU/kg was somewhat too small and FH was probably a little overestimated. The 30 and 50 mU/kg doses were given only at normoglycemia. FH was significantly ($P < 0.001$) smaller at hyper- than at normoglycemia also when these doses were excluded from this comparison.

The endogenous portal and arterial insulin levels during the IVGTT are compatible with a reduced FH at hyperglycemia. During the first few min the difference between the portal and arterial concentrations was indeed so small (Fig. 7) as to suggest a release of insulin from the liver. Such a possibility is suggested by the rapid and transient reversal of arterial-venous insulin gradients that occurs across the human forearm after local intraarterial injection of glucose (27). In addition, the initially small insulin gradients during the IVGTT may have been due to a number of other factors, such as a change in blood flow, the influence of proinsulin, and inaccuracy of portal sampling. Of these, the transient but substantial increase in portal plasma flow immediately after a glucose pulse (10) has an unequivocal effect. Proinsulin, which reacts with our insulin antiserum, is taken up by the liver to a smaller extent than is insulin (29). But proinsulin is only a small fraction of total immunoreactive insulin in portal and peripheral blood, in particular early after a glucose load (19), which indicates that it contributes little to the findings in Fig. 7. Though sampling from a portal catheter is sometimes subject to large random variation (15), averages of the results seem to be valid (10, 15).

Our finding that the fractional hepatic uptake of insulin decreases with increases in insulin and/or glucose agrees with some previous studies in vivo (11, 12, 22) but disagrees with others (8, 9, 15, 21). The divergent findings may, perhaps, be due to differences in experimental conditions. In studies with hepatic venous sampling, the most commonly employed technique (8, 11, 12, 15, 21), the results obtained seem to vary with the route of insulin supply. Thus, when the portal insulin level is raised above the arterial, by intravenous or intraduodenal infusion of glucose (8, 21) or by infusion of insulin (15), the fractional hepatic uptake of insulin increases (8, 21) or is essentially constant (15) as the insulin supply to the liver increases. Conversely, when

receptors by insulin was responsible. Studies in vivo (15, 30) and in vitro (24, 29, 33) with insulin concentrations as low as those obtained by the glucose infusions have shown no effect on hepatic uptake within 1-3 h. Down regulation of insulin receptors by insulin is a process which requires more time, especially when insulin concentrations are physiologic or moderately higher (20). It has also been suggested that down regulation has no physiological relevance (20). The absence of a correlation between FH and pretest endogenous insulin levels also argues against a role for occupancy alone.

The total hepatic uptake of insulin, i.e., the uptake of both first pass and recirculating insulin, can only be very roughly estimated. Considering the results after the 5-10 mU/kg doses, which gave insulin concentrations within the physiologic range (37), and assuming that FH is representative of the fractional hepatic uptake of posthepatic insulin, the total hepatic uptake should have been about 60 % of the portal dose at normoglycemia and about half as much at hyperglycemia. These estimations assume no dose-dependence and a constant blood glucose level and are therefore very approximate. Whatever the exact figures, it is, however, obvious that the hepatic uptake is substantial both at normo- and at hyperglycemia, and that the liver can modify the distribution of plasma insulin, at least shortly after increases in insulin and glucose. Redistribution of endogenous insulin after a glucose load is consistent with the finding that the main part of the excess glucose is taken up by peripheral tissues (6, 35). It is also consistent with results showing that glucose rather than insulin is the main determinant of net hepatic glucose balance, provided there is at least a low constant hepatic insulin supply (4, 5). It sounds reasonable to assume that a large FH at low blood glucose levels will help to prevent hypoglycemia. The diversion of larger fraction of pancreatically released insulin to the periphery during hyperglycemia, and a smaller one during normoglycemia, agrees well with the metabolic demands in the non-fasting as well as in the fasting state (7).

Variation of the plasma insulin concentration in peripheral blood is generally believed to reflect variation of the pancreatic secretion rate alone. But the present study showed that also changes in the fractional hepatic uptake may be responsible. In accordance with previous observations (24, 29, 30), the effect of insulin itself on FH is small when insulin concentrations are "physiologic". It is obvious, however, that a raised blood glucose level leads to a clear-cut decrease in the fractional hepatic uptake of insulin irrespective of any direct effect of the concomitant rise in insulin secretion. It is not known whether this effect of glucose holds also for diabetics.

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Extrahepatic Splanchnic Metabolism of Insulin, Glucose, and FFA

Running title: Extrahepatic Splanchnic Metabolism

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Summary

Portal-arterial concentration differences of insulin, C-peptide, glucose and FFA were measured in unanaesthetized, nondiabetic man. After injection of insulin a small but significant amount of it was taken up by the extrahepatic splanchnic bed (ESB). The portal and arterial concentrations of C-peptide, but not the portal-arterial difference, started to decrease 20 min or more following elevation of plasma insulin slightly exceeding the physiologic range. After insulin or glucose injection, glucose was taken up by the ESB, whereas the net uptake of FFA (the mean value being zero) varied negatively with the FFA decrement in arterial blood. In the fasting state the glucose exchange across ESB occurred in both directions, and sometimes net uptake or net release was predominant. It is suggested that the direction of net transport of glucose and FFA across ESB is adapted to the overall metabolic situation in the body.

Key words: Portal-arterial difference, extrahepatic splanchnic bed, insulin, C-peptide, glucose, FFA, human.

The extrahepatic splanchnic bed (ESB) receives about 20 per cent of the cardiac output in man, implying that any metabolic activity in this area may have a profound influence on the organism as a whole. Previous studies on the ESB exchange of insulin¹ (1-4), glucose (5-9) and FFA (6,10-13) have been performed in animals. The results have not been unaminous. In essence, these studies have demonstrated either an uptake or no net exchange across the ESB, whereas a few (3,4,9,13) have suggested that also a release can occur. In turn, lack of unequivocal evidence of an exchange of these compounds across the ESB has led to the view that hepatic venous-arterial differences fully represent hepatic exchange and that portal-arterial differences represent pancreatic insulin release alone.

Portal vein catheterization for portography (14) gave us the opportunity to measure extrahepatic splanchnic gradients of insulin, glucose and FFA in unanaesthetized humans in the fasting state and after injections of insulin and glucose.

Material and Methods

Subjects

The material consisted of 45 surgical ward patients (Table 1) with a transumbilical portal catheter (14). Most of them had carcinoma of the colon, but none had demonstrable metastases in the liver or elsewhere.

Table 1. Clinical characteristics

n	Sex		Age years	Body weight % of ideal ^a	Diagnosis	
	M	F			carcinoma ^b	Crohn's disease
45	29	16	61.4 ± 11.0 (32-82)	107 ± 12 (86-126)	39 (4)	2

First line gives means ± SD and second line gives the ranges

^aTables of Metropolitan Life Insurance Company

^bCarcinoma of the colon or, shown within parentheses, of the urinary tract

¹As far as insulin is concerned, the term ESB is to be understood as comprising the extrahepatic and the extrapancreatic bed.

All had normal fasting blood glucose concentrations. No patient was treated with drugs known to disturb glucose or insulin metabolism and none had endocrine disease. Portography for liver metastases was performed 2 days after portal catheterization (14) and 1 day before the experimental studies. The day after portal catheterization the patient resumed his preoperative routine, moved about freely in the ward and had an ordinary hospital diet. The portal catheter was kept patent by continuous infusion of heparinized saline (5000 IU of heparin in 1000 ml 154 mmol/l NaCl each day).

The investigations were performed in accordance with the principles embodied in the Declaration of Helsinki. Patient information and consent were verbal.

Experimental Protocol

The handling of the patients and the experimental procedures have been described elsewhere (15). After an overnight fast the heparinized solution was replaced by saline, which was infused at a rate of 1.6 ml/min throughout the experiment. One hour before the start of the study, peripheral plastic catheters were inserted, one into a radial artery for blood sampling and, in some instances, another into a contralateral cubital vein for injection.

In 25 patients, insulin Actrapid (monocomponent porcine insulin at neutral pH; Novo A/S, Copenhagen), diluted in 10 ml of saline, was injected into a peripheral ($n = 12$) or the portal ($n = 13$) vein for 2.5 min. Three dose levels were used, 8, 17 and 25-42 mU/kg ($n = 6, 8$ and 11, respectively). Five patients served as controls and received only 10 ml of saline intraportally. A standard intravenous glucose tolerance test (IVGTT, $n = 18$) was performed by giving 25 g of glucose in 100 ml of water into the peripheral vein during 2 min. Following insulin or glucose injection, samples (2 ml) were taken at -10, -5, 0, 2, (3,) 4, (5,) 6, 9, 12, 16, 20, 25, 30, (40,) 50, 60, (70, 80 and 90) min (figures within parentheses denote times at which blood was not collected in all cases). Portal blood was sampled 10 sec after the arterial.

Since the experimental procedure varied with respect to route of insulin infusion and since all tests were not done in every patient, Table 2 shows the number of each type of study. In 15 patients fasting was prolonged to 14⁰⁰ p.m., at which time an IVGTT was performed. Otherwise, the tests started in the morning (confer Table 3).

Table 2. Number of experiments in each study group

	Expt. no.	Determination of portal and arterial			
		insulin	C-peptide	glucose	FFA
NaCl	1-5	5	0	5	5
Peripheral insulin injection (8-25 mU/kg)	6-17	12	8	12	4
Portal insulin injection (8-42 mU/kg)	18-30	0	4	13	9
IVGTT (25 g)	31-48	18	0	18	15

Analytical Methods

Test tubes contained EDTA. Blood glucose was immediately determined in triplicate with a glucose oxidase method (16). Plasma rapidly separated and stored at -20°C for later determination of FFA, insulin and C-peptide. Total immunoreactive insulin and C-peptide were determined in triplicate according to Heding (17,18). FFA were measured with the method of Trout et al (19). Details of our assay for insulin have been given elsewhere (15). The insulin assay does not distinguish between endogenous and exogenous (porcine) insulin. The C-peptide was assayed with reagents purchased from Novo Research Institute, Copenhagen. The sensitivity of the C-peptide assay is about 0.05 pmol/ml and the within-assay coefficient of variation is 17 per cent. In this assay proinsulin contributes less than 3 per cent to the measured C-peptide. The within-assay coefficient of variation for respectively glucose and FFA is 2.5 and 2 per cent. In order to minimize analytical variation, all samples from one patient were determined in a single assay run.

Calculations

The arterial-portal differences (APD:s) were evaluated according to the principles laid down by Zierler (20). In the basal state exchange across ESB was estimated by the (almost) simultaneous, paired APD:s. (Assumptions: constant concentration, constant flow, constant uptake.) Otherwise, exchange across ESB was estimated by comparing the integrals of the arterial and portal concentration curves, i.e. by the integrated APD:s. The insulin uptake across ESB during hyperinsulinemia was estimated by integration of APD from zero time up to the time at which the portal insulin concentration had returned to its pretest value.

As for the effect of insulin on glucose and FFA, the values after injection of insulin were pooled in the following way. Since only those insulin molecules reaching the systemic circulation can affect ESB exchange, we determined the increase in plasma insulin concentration that would have been obtained in the systemic circulation if all insulin had remained in the plasma pool. This estimate is called SER (simulated early insulin response) ($\mu\text{U/ml}$) and was calculated from

$$\text{SER} = k_{e1} \cdot \int_0^{\infty} I_a(t) dt \quad (1)$$

where the integral and k_{e1} (min^{-1}), the net fractional transfer rate constant from the plasma to extravascular pools, were calculated as previously described (15). I_a ($\mu\text{U/ml}$) is the arterial plasma insulin concentration above baseline. SER may thus be regarded as an estimate of the posthepatic or systemic dose normalized by the plasma volume.

Areas were calculated by trapezoidal integration. Owing to the findings in the fasting state, APD changes after insulin or glucose were estimated after subtraction of the basal APD value. After glucose, the tail areas of the glucose concentration curves were calculated by exponential extrapolation unless the pretest value had been reached. The rate constant (min^{-1}) of the IVGTT was calculated as proposed by O'Sullivan et al (21).

The effect of insulin on arterial glucose or FFA was calculated as the decrement from the basal level to the mean of the two lowest consecutive concentrations recorded. Blood glucose decrement (BGD) and plasma FFA decrement (FD) were expressed as percentual changes from the basal level.

Statistical Methods

Non-parametric tests were used (22). Thus, differences between independent samples were tested with the Mann-Whitney or the Kruskal-Wallis test, and differences between matched-pairs with the Wilcoxon test. Correlation was estimated by the Spearman rank correlation coefficient (r_s).

Results

Basal Values

Table 3 summarizes the fasting values. The mean APD:s for glucose and FFA did not differ from zero and did not vary during the day. But the repeated measurements in the same individual showed that the APD for glucose was relatively stable within each patient, and that it differed significantly between patients ($p < 0.001$). In the afternoon APD varied with the arterial blood glucose level ($r_s = 0.70$, $p < 0.01$), i.e. the lower the blood glucose concentration the smaller the APD. In contrast,

Table 3. Basal concentrations

	Time of day	n	Arterial	Portal	Arterial-portal difference (APD)
Insulin, $\mu\text{U/ml}$	8 ⁰⁰	30	11(6-16)	22(14-34)	-10(-5 - -19)
	14 ⁰⁰	15	4(3-9)	8(4-15)	- 5(-2 - -9)
C-peptide, pmol/ml	8 ⁰⁰	12	0.15(0.12-0.42)	0.17(0.15-0.54)	-0.04(-0.01 - -0.09)
Glucose, mmol/l	8 ⁰⁰	30	4.15 $\pm$ 0.57	4.15 $\pm$ 0.59	0.00 $\pm$ 0.12 ^a
	14 ⁰⁰	15	4.21 $\pm$ 0.63	4.19 $\pm$ 0.53	0.03 $\pm$ 0.21 ^a
FFA, $\mu\text{mol/l}$	8 ⁰⁰	17	323 $\pm$ 86	325 $\pm$ 82	-2 $\pm$ 11 ^a
	14 ⁰⁰	12	464 $\pm$ 64	464 $\pm$ 67	1 $\pm$ 10 ^a

Insulin and C-peptide values are medians and 20th and 80th percentiles, glucose and FFA values are means $\pm$ SD

^aValues not differing from zero ($p > 0.05$)

Table 4. Effect of insulin on arterial blood glucose and plasma FFA

	Peripheral insulin (mU/kg)			Portal insulin (mU/kg)		
	8	17	25	8	17	25-42
BGD	8 $\pm$ 5(3)	17 $\pm$ 4(6)	22 $\pm$ 3(3)	11 $\pm$ 2(3)	15 $\pm$ 2(2)	23 $\pm$ 9(8)
FD	6 $\pm$ 2(2)	13 (1)	46 (1)	23 $\pm$ 20(3)	66 (1)	36 $\pm$ 16(5)

Values are means $\pm$ SD

BGD = arterial blood glucose decrement, FD = arterial FFA decrement, both as percentages of the pretest value (see Calculations).No. of expts. within parentheses

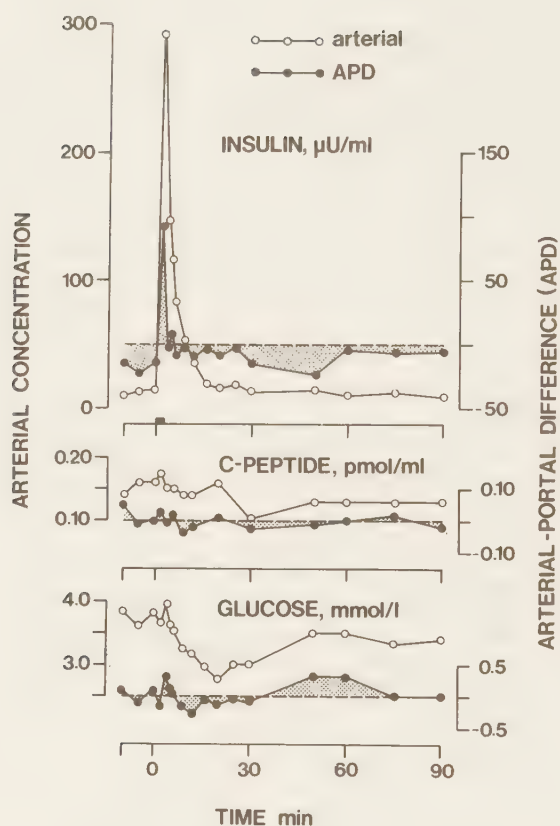


Fig. 1. Arterial concentrations and arterial-portal differences (APD:s) of insulin, C-peptide and glucose in a patient receiving 17 mU/kg of insulin into a peripheral vein. Filled bar denotes the insulin infusion. Baseline values have not been subtracted

Table 5. Uptake of insulin and glucose across the extrahepatic splanchnic bed (ESB) after peripheral injection of insulin

Dose mU/kg	n	Insulin uptake ^a $\mu\text{U min ml}^{-1}$	Glucose uptake ^a mmol min l^{-1}
8	3	100 ± 161	3.00 ± 8.01
17	6	303 ± 164	-0.82 ± 10.92
25	3	341 ± 268	6.25 ± 10.27

Values are means $\pm$ SD

^a Estimated as described in Calculations. Positive sign denotes uptake

the APD for FFA did not vary between patients or with the arterial concentration. Systemic and portal FFA concentrations were higher in the afternoon than in the morning whereas blood glucose showed no such variation.

In the 5 patients receiving saline alone (expts. 1-5), glucose and FFA concentrations were stable and APD varied between patients both with respect to glucose ($p < 0.01$) and FFA ($p < 0.05$). Four of these patients showed a significant uptake of glucose across ESB, the mean APD being 0.11 mmol/l.

Insulin Injection

Glucose and FFA concentrations invariably decreased after injection of insulin, roughly in proportion to the dose given (Table 4). Fig. 1 gives a typical set of data following peripheral injection of insulin.

Plasma insulin (expts. 6-17). The results following peripheral insulin injection are summarized in Table 5. There was an uptake of insulin across ESB ($p < 0.01$). It was lowest in the 8 mU/kg dosage group, and more than three times as high when the dose was 25 mU/kg. However, the correlation between ESB uptake and dose, or SER, did not reach statistical significance ($p > 0.05$). Assuming a portal plasma flow of $10 \text{ ml min}^{-1} \text{ kg}^{-1}$ (23), the uptake corresponded to 15 per cent of the dose given.

Plasma C-peptide (expts. 6-30). In no case were consistent changes in APD for C-peptide observed. On the other hand, both portal and arterial C-peptide started to decrease at 20-30 min, with maximum decrements at 30-80 min, after the larger insulin doses. The decrease tended to be correlated with dose or BGD, though C-peptide invariably decreased only when BGD was 15 per cent or more. C-peptide did not decrease after the smallest dose (8 mU/kg), but it did in 3 of 4 patients given 17 mU/kg (one of these cases is illustrated in Fig. 1) and in all patients receiving larger doses. After the 17-42 mU/kg doses, the maximum decrease in C-peptide was, on the average, 37 per cent. C-peptide did not start to decrease as long as the portal insulin concentration remained above its pretest value. After that the duration of the C-peptide decrease was highly variable and the C-peptide level showed no consistent relationship to the concomitant insulin or glucose concentration.

Blood glucose (expts. 6-30). Insulin injection was followed by a significant uptake of glucose across ESB ($p < 0.05$). After peripheral insulin (expts. 6-17), the glucose uptake varied with the insulin uptake (Fig. 2; $r_s = 0.68$, $p < 0.05$), but not significantly with the insulin dose (Table 5, $p > 0.05$). Similarly, the uptake of glucose did not vary with SER when the results of all experiments were pooled (expts. 6-30, Fig. 3). Yet it is noteworthy that the data (Fig. 3) suggest that small increases in plasma insulin were accompanied by an uptake of glucose, whereas large increases were accompanied by no net exchange or by a pronounced excursion of net glucose transport in either direction. BGD was maximal at 20-50 min. The data did not indicate that the exchange

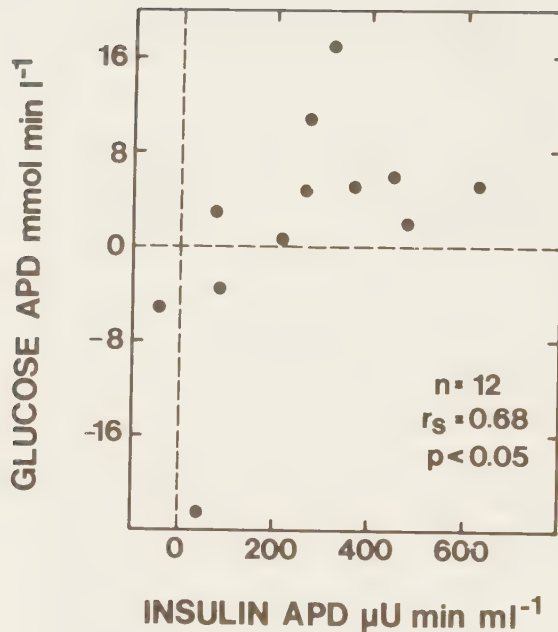


Fig. 2. Relationship between the integrated arterial-portal differences (APD:s) for plasma insulin and blood glucose following peripheral injection of insulin. A positive APD denotes uptake

of glucose across ESB was in opposite directions before and after the arterial blood glucose nadir.

Plasma_FFA_(expts. 6-30). The average integrated APD value for FFA after injection of insulin was 0.16 ± 1.16 (mean $\pm$ SD) mmol min l⁻¹, not different from zero ($p > 0.05$). However, as shown in Fig. 4, the APD area varied negatively with SER ($r_s = -0.60$, $p < 0.05$), i.e. the larger the systemic insulin dose the smaller the value of the APD area. Similar correlations were found for the APD area of FFA versus BGD ($r_s = -0.58$, $p < 0.05$) or FD ($r_s = -0.62$, $p < 0.05$). In those cases where the APD area indicated uptake, this started during the development of the hypolipacidemia and, in all but one case, continued after the arterial FFA nadir. In contrast, in those cases where the APD values indicated release from the ESB, this occurred only during the period of recovery from the hypolipacidemia, whereas neither uptake nor release appeared to take place before the arterial FFA nadir (at 20-30 min).

IVGTT

After glucose injection (expts. 31-48) the integrated APD for glucose increased 22.9 ± 31.2 (mean $\pm$ SD) mmol min l⁻¹ ($p < 0.01$). The uptake

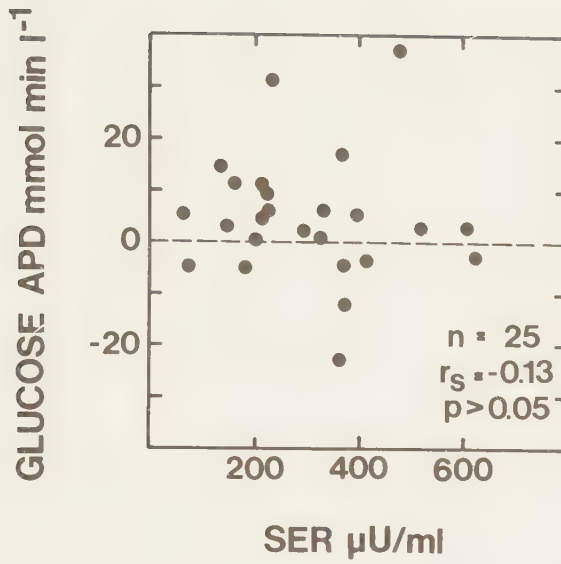


Fig. 3. Integrated arterial-portal difference (APD) for glucose versus the simulated early insulin response (SER), the latter variable representing the net total insulin rise after insulin injection (see Calculations). A positive APD represents uptake

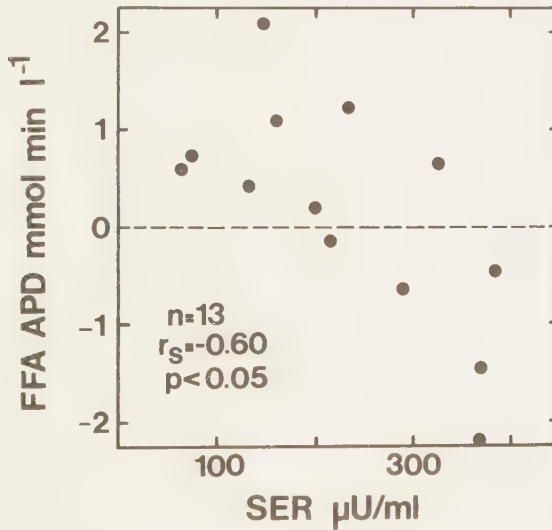


Fig. 4. Integrated APD for FFA versus SER. See legend to Fig. 3

of glucose corresponds roughly to 20 per cent of the dose given (23). On the average, no change in APD for FFA could be detected, but like the findings after insulin, there was a wide interindividual variation and, again, the APD area varied negatively with the arterial FFA decrement ($r_s = -0.59$, $p < 0.05$), the latter calculated as after insulin. Arterial and portal concentrations of FFA decreased markedly.

Discussion

This study showed that the ESB takes up insulin. It also demonstrated that the ESB takes up and releases glucose and FFA, and indicated that the direction of net exchange of these compounds depends on the prevailing metabolic situation.

The estimation of exchange across ESB involves the calculation of a small difference (APD) between large numbers. By calculating integrated APD:s the experimental error should be minimized. This is particularly important in the non steady-state where the timing of blood samples may otherwise constitute a difficult problem (20). When C-peptide decreased, it did not start to do so before the portal insulin concentration had returned to its pretest value, which was the end-point of the measurements of insulin APD. We therefore believe that the estimated insulin uptake was not at all, or only minutely, influenced by changes in the endogenous secretion rate. As illustrated in Fig. 1, the differences between the portal and arterial concentrations of insulin were often large during the first 3 min. Considering the 1-1.5 min necessary for good intravascular mixing (24), it is possible that these large initial differences were partly only apparent. (The use of a mean transit time of 20 (25) instead of 10 sec would decrease the areas only slightly.) Anyhow, when only samples obtained after 3 min were used for the calculations, the uptake was about one third as high but still significant ($p < 0.05$). The relationship between the balances of insulin and glucose across ESB (Fig. 2) is further evidence that insulin is taken up and also evidence that it is metabolically active in the ESB.

Our estimate of the insulin uptake across ESB (Table 5) corresponded to about 15 per cent of the dose given, implying that the fractional extraction was in the range of 20-25 per cent (see Appendix). As pointed out above, this figure should be regarded as the maximum possible. On the average, a fractional extraction ratio of 20 per cent, or somewhat less, agrees with figures obtained in animals though the variation has been considerable (1-4).

In the fasting state the APD for glucose was zero when the results in all patients were pooled. But the differences between patients indicated that the ESB was taking up glucose in some of them and releasing it in others. When fasting was prolonged until the afternoon, the APD value varied with the systemic blood glucose concentration. Since the mean APD value was zero, this indicated that glucose may be released from the ESB when blood glucose is low and taken up when it is high. During

prolonged fasting it is likely that the supply of glucose from the liver becomes gradually reinforced by supply from the ESB. This may explain why only the afternoon studies showed a relationship between the systemic concentration and the APD value for glucose.

Most previous studies in fasting animals have shown either a net uptake (5,6) or no net exchange (7,8) of glucose across the ESB, the absence of net exchange extending to insulin and/or glucose infusion (7,8). However, the finding that glucose transport across the ESB can occur in both directions is supported by one earlier study in dogs (9) and also by the following. Our studies clearly demonstrated an uptake of glucose from the blood in response to insulin or glucose injection. Moreover, studies in vitro have shown that the intestinal mucosa takes up sugars reaching the enterocytes from the basal side (26). Ability of the ESB to release glucose is of course compatible with the fate of ingested glucose. In this respect it should also be observed that Fig. 3 suggests that the action of insulin on the ESB may be counterbalanced or overcome by alterations secondary to the hypoglycemia provided that the insulin dose is large and blood glucose is low (confer FFA). As discussed previously (27), changes in the direction of net glucose transport across the intestine may explain some features of the portal and arterial concentration curves obtained after oral glucose.

There was no average exchange of FFA across ESB when the experiments were pooled. As for the net balance of total FFA across ESB, our results thus agree with those of others (10-13). However, it has been shown that labelled palmitate is removed from the ESB (10,13) without any simultaneous change in the APD value for total FFA, inferring that FFA is also released from this area, and that the intestinal wall can take up and release FFA in vitro (28). An active role for ESB in the metabolism of FFA is indeed compatible with some of our own observations. The relationships between the APD for FFA, on one hand, and SER, BGD or FD, on the other, suggest that there may be a net release of FFA from the ESB when the utilization of, and the need for, FFA is increased and systemic levels are low. In other words, the larger the net uptake of FFA (by muscle) and the larger the body uptake of glucose the larger the net release of FFA from the ESB. Such an interpretation is consistent with findings during exercise, where increased uptake of glucose and increased net uptake of FFA (by muscle) occur concomitantly with a net release of FFA from the splanchnic area, probably its extrahepatic part (29). Furthermore, the positive APD values for FFA after the small insulin doses (Fig. 4) suggest that insulin itself inhibits FFA release from the ESB provided no secondary, compensatory changes overcome its effect.

Our results suggest that the direction of net transport of glucose and FFA across the ESB depends on the overall metabolic situation in the body. The evidence is that the ESB exchange of glucose varied with arterial blood glucose in the afternoon, and that the ESB response to insulin for glucose and FFA varied with the insulin dose. Glucose and FFA were taken up when the effect of insulin was small, and released

when it was large and they were needed in other tissues. After insulin injection, it thus appears that the net exchange across ESB of glucose and, especially, FFA depends on the action of insulin on ESB as well as the secondary changes induced by insulin in the body. As for glucose, the response of the ESB to insulin dose resembles that of the liver (e.g. ref. 9) but differs from that of other nonhepatic tissues. As for FFA, the response differs both from that of the liver (12) and other organs.

Finally, our results show that total and hepatic splanchnic metabolism cannot generally be equated. Arterial-hepatic venous differences are influenced by an exchange across ESB that may vary both between patients as well as within patients in different metabolic situations.

Appendix

The following derivation allows an approximate estimation of the fractional extraction ratio of insulin across ESB, i.e. of $(I_a - I_{po})/I_a$ (I_a and I_{po} are the arterial and portal concentrations of plasma insulin). Denote the total ESB uptake as a fraction a of the peripheral dose D ($\mu\text{U/kg}$), the portal plasma flow by PPF ($\text{ml min}^{-1}\text{kg}^{-1}$), and the fractional extraction ratio by b . Then:

$$a \cdot D = b \cdot \text{PPF} \cdot \int_0^{\infty} I_a(t) dt \quad (2)$$

The integral $\int_0^{\infty} I_a(t) dt$ is equal to D/PCR , where PCR is the plasma clearance rate ($\text{ml min}^{-1}\text{kg}^{-1}$) of the injected insulin. Substitution and rearrangement gives that

$$b = a \cdot \text{PCR} / \text{PPF} \quad (3)$$

PCR and PPF are about 15 and $10 \text{ ml min}^{-1}\text{kg}^{-1}$, respectively (15,23). If a is assumed to be 0.15 (see Results), b will be 0.225 .

As for the ESB uptake of glucose during the IVGTT, a similar calculation will show that the fractional extraction ratio is about 0.05 .

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Variation of Plasma Disappearance of Unlabeled Insulin: Studies with
Portal and Peripheral Infusions

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Running title: PLASMA INSULIN DISAPPEARANCE IN MAN

SUMMARY

One hundred and thirty brief infusions of unlabeled insulin were given to 75 unanesthetized nondiabetic humans. The patients were examined (a) by different doses of insulin (5-50 mU./kg.), (b) by intraportal and peripheral infusion, (c) in the morning and in the afternoon, and (d) during normoglycemia and moderate steady hyperglycemia. After intra-portal infusion of insulin, the non steady-state plasma clearance rate (PCR, ml. min.⁻¹ kg.⁻¹) was lower the larger the dose (decreased about 50 per cent by a tenfold increase in insulin dose) and lower at hyper- than at normoglycemia (decreased about 30 per cent after small insulin doses). It was also lower the higher the relative body weight or the older the patient, but these two correlations were weak. After peripheral insulin infusion none of the above relationships was demonstrated. Regardless of route of insulin infusion, PCR did not vary with the fasting plasma insulin concentration, the time of the day, the insulin sensitivity or the glucose tolerance. It is concluded that the liver modifies the distribution and removal of pancreatically released insulin in response to acute increases in insulin or glucose. But otherwise insulin removal showed a notable lack of relationships with common indices of metabolism and with insulin action.

The current concept of insulin receptor regulation by insulin¹ has revived the interest in factors related to insulin metabolism and its coupling with insulin action. It has also brought into focus the question to what extent the plasma insulin concentration is determined by the rate of disappearance. But studies in vivo are few and the results are not unanimous.²⁻⁶ Furthermore, in humans disappearance of insulin entering the circulation via the "physiological" portal route has been studied only with labeled insulin during anesthesia.⁷

Having the opportunity to investigate patients on whom portal catheterization was performed for possible liver metastases,⁸ we studied the disappearance of insulin from plasma after a brief infusion of insulin into the portal vein. In a previous report,⁹ we analysed the kinetics of insulin removal from plasma. The present paper describes how the clearance of insulin is related to variables such as plasma insulin, blood glucose, insulin sensitivity, glucose tolerance, relative body weight and the time of the day. The results were also compared with those obtained after infusion of insulin into a peripheral vein in order to estimate the role played by the liver.

MATERIAL AND METHODS

Subjects. The material consisted of surgical ward patients with normal fasting blood glucose concentrations. None had previous endocrine disease and none was receiving any drug known to disturb insulin or carbohydrate metabolism. Body weights ranged from 50 to 97 kg. and from 86 to 141 per cent of the ideal (Tables of Metropolitan Life Insurance Company). The results of standard liver and kidney laboratory tests were normal in all.

Fifty-eight patients with a transumbilical portal catheter⁸ were given intraportal infusions of insulin. In addition, 39 of them received a peripheral insulin infusion, as did another 17 patients. Since the experimental procedure varied with respect to route of insulin infusion, time of day and glucose level, the clinical characteristics of each study group are summarized in table 1. Most patients with a portal catheter were to be, or had been, treated for carcinoma of the colon, whereas patients receiving only peripheral infusions of insulin had a variety of diagnoses, mostly inguinal hernia. There was otherwise no difference in basic clinical or laboratory data between the study groups or between patients with and without a portal catheter (table 1). Patients with portal catheters were examined for liver metastases by means of portography,⁸ liver scintigraphy, and subsequent laparotomy. Only patients without demonstrable metastases or portal-systemic shunting were included. No study was performed within 6 months after major operation. In 6 patients undergoing inguinal herniorrhaphy, studies were performed also 3 days postoperatively. The results of these postoperative studies were excluded from the rest and are given separately.

TABLE 1

Clinical characteristics of each study group

Route of insulin infusion	Time of infusion day	Glucose infusion	No. of subjects*	Sex (M/F)	Age (years)	Relative weight (% of ideal)	Fasting blood glucose (mmol./l.)	Fasting plasma FFA (μ mol./l.)	Fasting plasma insulin (μ U./ml.)	k_G (%/min.)	Diagnosis	Miscellaneous
Portal	8.00	-	32(36)	20/12	63.0 $\pm$ 8.1	112 $\pm$ 12	4.32 $\pm$ 0.55	388 $\pm$ 90	12.0(5.6-20.0)	0.95 $\pm$ 0.25	28 (4)	
	8.00	+	16(16)	7/9	67.6 $\pm$ 5.5	111 $\pm$ 11	4.41 $\pm$ 0.34	358 $\pm$ 100	12.0(3.0-16.0)	1.13 $\pm$ 0.27	13 (2)	1
	14.00	-	10(10)	6/4	61.4 $\pm$ 7.1	109 $\pm$ 10	3.90 $\pm$ 0.48	490 $\pm$ 69	3.0(1.2- 7.0)	0.89 $\pm$ 0.15	10	
Peripheral (Peripheral only)	8.00	-	41(53)	29/12	62.0 $\pm$ 10.7	111 $\pm$ 12	4.22 $\pm$ 0.51	361 $\pm$ 106	10.0(5.0-17.0)	0.95 $\pm$ 0.27	20 (4)	10
			17(27)	14/3	60.1 $\pm$ 13.1	109 $\pm$ 11	4.26 $\pm$ 0.42	395 $\pm$ 157	10.0(4.6-21.0)	1.07 $\pm$ 0.25	7	10)
	8.00	+	15(15)	7/8	67.3 $\pm$ 5.6	111 $\pm$ 11	4.52 $\pm$ 0.59	369 $\pm$ 124	11.0(7.0-13.8)	1.12 $\pm$ 0.28	12 (2)	1

The plasma insulin values are medians and 20th and 80th percentiles, otherwise the values are means $\pm$ SD.

* No. of insulin infusions are given within parentheses.

† Neoplasm of the colon or, when in parentheses, of the urinary tract.

‡ Malignant disease 4, nephrolithiasis 3, sigmoiditis 3, gastritis 1, crural varices 1.

The portal catheter had been inserted under general anesthesia, 2 days before portography, and 3 days before the experimental studies. The day after catheterization the patient resumed his preoperative routine, walked about freely in the ward, and was on an ordinary hospital diet. The portal catheter was kept patent by continuous infusion of 5000 I.U. of heparin in 1000 ml. of saline each day.

The investigations were performed in conformity with the principles laid down in the Declaration of Helsinki. Verbal informed consent was obtained from all participants.

Experimental procedures. The experimental procedures have been described in detail elsewhere.⁹ The studies were begun after an overnight fast, 1 hour after discontinuation of the portal infusion of heparin and cannulation of a radial artery for blood sampling. When the patient was to receive a peripheral infusion, a contralateral cubital vein was also catheterized. When glucose was infused, it was given via the portal catheter throughout the experiments, and then the pretest period was extended 90 (during infusion of $80 \text{ mg. kg.}^{-1} \text{ h.}^{-1}$) or 120 (during infusion of $200 \text{ mg. kg.}^{-1} \text{ h.}^{-1}$) min. to ensure steady levels of glucose and insulin before the infusion of insulin.⁹ The infusions of glucose raised the arterial blood glucose levels by 0.89 ± 0.46 and $2.32 \pm 0.82 \text{ mmol./l.}$, respectively, and the plasma insulin levels by 4.0 ± 6.4 and $21.6 \pm 21.2 \text{ } \mu\text{U./ml.}$ During glucose infusion, portal blood glucose concentration was about 0.4 and 1 mmol./l. higher than that in arterial blood. Unless otherwise stated, studies were begun in the morning. In 10 patients fasting was prolonged to 2 p.m. before the studies began. In patients examined twice, the second study was performed 1 day after the first.

Insulin Actrapid (monocomponent porcine insulin at neutral pH; Novo A/S, Copenhagen) was infused for 2.5 min. and samples were taken at frequent intervals from a radial artery during the following hour. An intravenous glucose tolerance test (IVGTT) was begun 60 min. after start of the insulin infusion by giving 25 gm. of glucose in 100 ml. of water into a peripheral vein during 2 min. Arterial blood for glucose determination was sampled 72, 80, 90, 110 and 120 min. after the beginning of the insulin infusion (time 0).

Analytical methods. Sampling tubes contained EDTA. Blood glucose was measured in triplicate with a glucose oxidase method,¹⁰ the within-assay coefficient of variation being 2.5 per cent at a level of 3.70 mmol./l. Plasma was rapidly separated and stored at -20°C. until determination of total insulin immunoreactivity¹¹ and FFA.¹² Details of the insulin assay have been presented elsewhere.⁹ The within-assay coefficient of variation for FFA was 2 per cent.

Calculations. The disappearance of insulin from plasma was previously analysed in detail with emphasis on the computations, the assumptions and the limitations of the equations employed.⁹ Plasma clearance rate, PCR ($\text{ml. min.}^{-1} \text{ kg.}^{-1}$), was calculated as dose divided by the area of

the incremental plasma insulin concentrations, i.e., as $PCR = D / \int_0^{\infty} IRI(t) dt$, where D ($\mu U./kg.$) is the dose of insulin and IRI ($\mu U./ml.$) is the immunoreactive insulin concentration above the pretest baseline in arterial plasma.* Since this calculation is independent of the complexity of the disappearance, PCR is the least controversial estimate of plasma disappearance rate. After portal infusion the first pass hepatic uptake of insulin was treated as an instantaneous loss (see figure 1); which means that PCR_{po} includes the corresponding clearance rate. For insulin reaching the systemic circulation, compartmental analysis (figure 1) was used to estimate k_{e1} (1/min.), the average net fractional transfer rate constant from the apparent plasma volume, APV ($ml./kg.$). In the two-compartment model with irreversible loss from the extravascular compartment, k_{e1} is equal to $k_{21} \cdot k_{32} / (k_{12} + k_{32})$. If it is assumed that irreversible exit is from the plasma pool (see figure 1), k_{e1} ($= k_{31}$) will be identically large. The relationship $APV = PCR/k_{e1}$ was then utilized to calculate APV.⁹ The reason for calculating APV is as follows. After peripheral infusion of insulin, APV_{pe} should equal the value of plasma volume, but after portal insulin infusion APV_{po} should not because the clearance rate corresponding to the first pass fractional hepatic uptake is included in PCR_{po} . It is evident that the larger the first pass fractional hepatic uptake of insulin the larger the APV_{po} value.

The loss of insulin due to adsorption to syringes and catheters was 14 - 16 per cent.⁹ The appropriate values were used in all calculations, but the uncorrected doses are given in the text.

The effect of insulin on arterial glucose or FFA was calculated as the decrement from the pretest level to the mean of the two lowest consecutive concentrations and was expressed as percentual change from the pretest level.¹³ The k_g value of the IVGTT was calculated as proposed by O'Sullivan et al.¹⁴

Statistical methods. Standard statistical formulas were used.¹⁵ Fasting plasma insulin was log transformed before statistical analysis because of skewness to the right. No other distribution was significantly skew, but log transformation was performed before statistical analysis when it gave distributions and residuals closer to the normal and stabilized the variances. Unless otherwise stated, values given are means $\pm$ SD.

*Abbreviations used in this paper: PCR, plasma clearance rate; k_{e1} , net fractional disappearance rate from systemic circulation; APV, apparent plasma volume. The subscripts po and pe denote respectively the portal and peripheral routes of infusion.

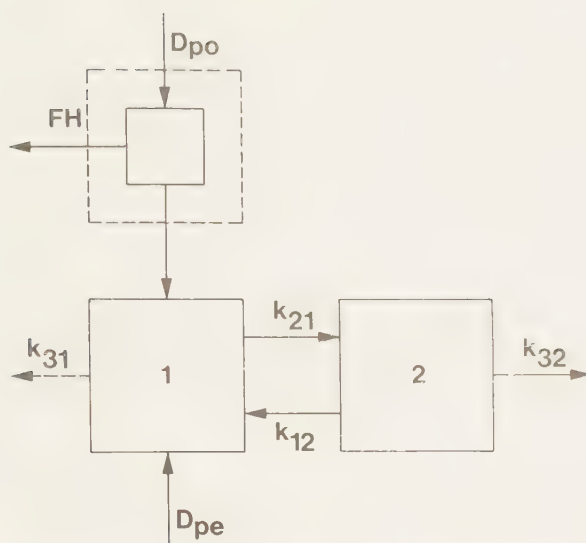


FIG. 1. Compartmental model for portal and peripheral infusion (D_{po} , portal dose; D_{pe} , peripheral dose).⁹ For portal infusion, the first pass fractional hepatic uptake is designated FH. After the first liver passage $(1-FH)D_{po}$ (the posthepatic dose) thus reaches the systemic circulation. The hepatic removal of posthepatic (recirculating), and peripherally administered, molecules is lumped together with the removal in peripheral tissues. For molecules entering the systemic circulation, the flow between intravascular (1) and extravascular (2) compartments has rate constants k_{21} and k_{12} and the irreversible loss has rate constant k_{32} (or k_{31}).

RESULTS

Representative sets of data for portal and peripheral infusions of insulin are given in figures 2-3. For most of the subjects studied detailed data on the modeling of the kinetics of insulin have been reported previously.⁹ In this paper we only summarize the values of PCR (table 2) and of APV (figure 4). The effect of insulin on arterial blood glucose and plasma FFA concentrations is given in table 2. At steady hyperglycemia the two dose levels of glucose infusion gave similar results, which were therefore pooled. It may be added that insulin kinetics, insulin sensitivity or glucose tolerance did not differ between patients with and without a portal catheter.

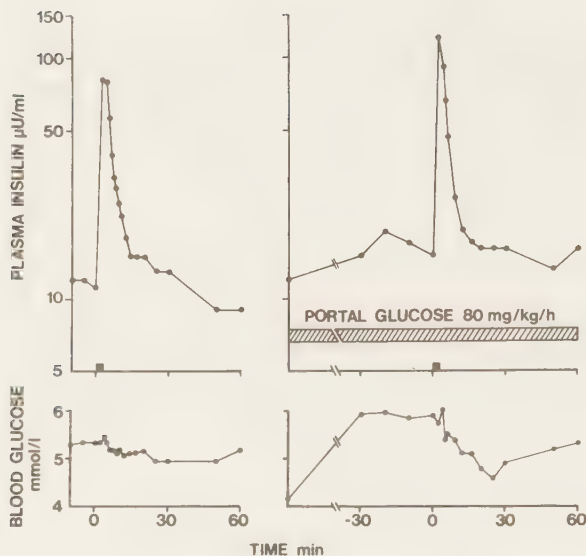


FIG. 2. Time-course of arterial plasma insulin and blood glucose before and after intraportal infusion of 10 mU./kg. at normoglycemia (left) and at steady hyperglycemia (right). Filled bar denotes the insulin infusion. Plasma insulin values are logarithmic.

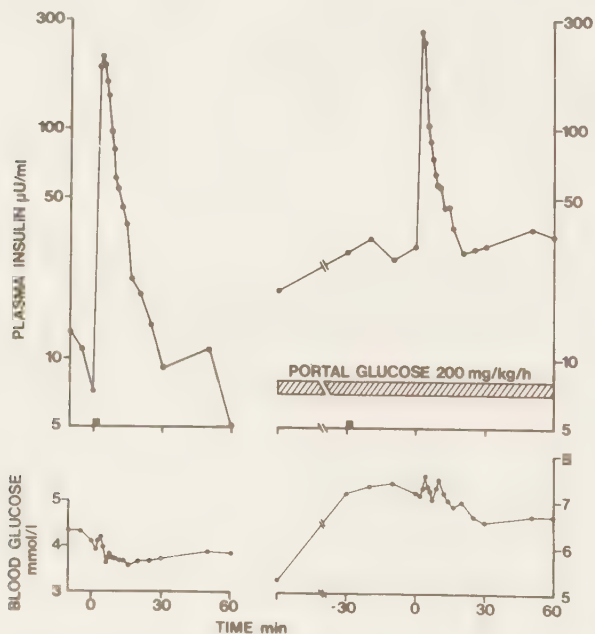


FIG. 3. Plasma insulin and blood glucose concentrations before and after peripheral infusion of 20 mU./kg. at normoglycemia (left) and steady hyperglycemia (right). See legend to figure 3.

TABLE 2

Plasma clearance rate and effect of insulin as functions of insulin dose

Time of day	Glucose level	Dose (mU, kg. ⁻¹)	Portal infusion			Peripheral infusion		
			PCR (ml. min. ⁻¹ kg. ⁻¹)	Blood glucose decrement (%)	Plasma FFA decrement (%)	PCR (ml. min. ⁻¹ kg. ⁻¹)	Blood glucose decrement (%)	Plasma FFA decrement (%)
a.m.	N	5	32.1±11.7(5)	9.1±1.0(3)		15.4±5.0(11)	7.6±2.6(7)	12.3±15.3(5)
		10	30.0± 9.0(11)	11.7±2.4(10)	9.4±8.0(7)	16.0±4.6(21)	11.6±4.7(16)	12.9±13.5(14)
		15				13.0±2.9(2)	15.1±4.2(2)	20.9±30.0(2)
		20	20.8± 4.2(7)	18.4±5.2(6)	13.0±12.3(3)	14.7±2.4(10)	18.2±6.3(10)	13.0±21.0(3)
		30	18.7± 7.0(5)	18.5±3.3(5)	19.1±20.0(3)	15.7±3.8(9)	22.0±3.5(6)	41.9 (1)
		40	17.0± 4.8(3)	21.2±2.8(3)	47.3 (1)			
		50	14.1± 2.8(5)	31.0±7.4(5)	38.8 (1)			
		5				16.6±3.6(5)	4.1±3.0(5)	
		10	21.0± 7.9(5)	9.8±8.5(5)				
		15				15.2±2.8(5)	10.5±2.8(4)	
p.m.	N	20	21.7± 5.3(7)	12.6±5.0(7)		15.4±1.2(2)	17.3±9.3(2)	
		30				11.4±2.1(3)	26.1±4.9(3)	
		40	13.8± 2.3(4)	27.2±2.1(3)				
		10	33.0±11.4(3)	8.8±3.2(3)	23.2±20.5(3)			
		20	26.7± 9.4(2)	15.0±2.4(2)	66.3 (1)			
		30	19.2± 4.4(5)	15.7±2.9(5)	31.7±17.1(4)			

Values are means ±SD. No. of determinations are given within parentheses. PCR, plasma clearance rate; N, normoglycemia; SSH, steady-state hyperglycemia.

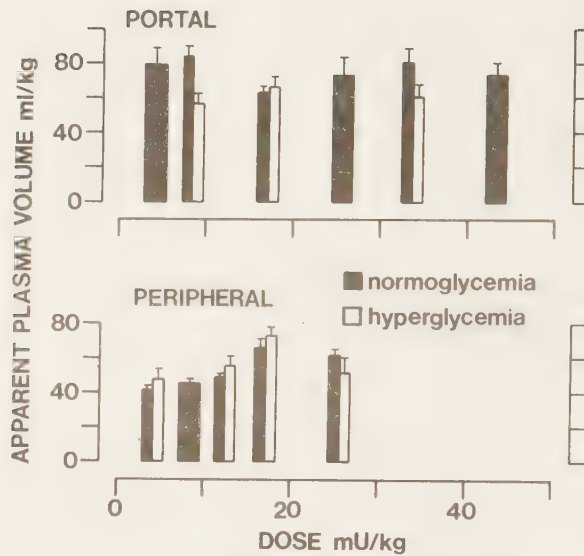


FIG. 4. Apparent plasma volume (APV) after portal and peripheral infusion of insulin. Values comprise the morning studies and are means $\pm$ SD.

Portal Insulin Infusion

PCR_{po} decreased markedly with increasing dose of insulin (table 2), but significantly so only at normoglycemia (normoglycemia: $r = -0.71$, $p < 0.001$; hyperglycemia: $r = -0.46$, $p < 0.10$). Hyperglycemia was associated with a decrease of PCR_{po} , too (table 2). Thus, after a dose of 10 mU./kg. PCR_{po} fell from 30.0 ± 9.0 at fasting to 21.0 ± 7.9 ml. min.⁻¹ kg.⁻¹ during glucose infusion ($p = 0.05$). But the effect of hyperglycemia on PCR_{po} was not apparent after larger doses of insulin. As a consequence, PCR_{po} was overall significantly smaller at hyper- than at normoglycemia only when morning and afternoon studies were pooled (analysis of covariance, $p < 0.05$). The PCR_{po} , or APV_{po} , values obtained in the afternoon did not differ from those obtained in the morning, despite the fact that endogenous plasma insulin was significantly ($p < 0.01$) lower when fasting was prolonged to the afternoon (table 1). APV_{po} did not vary with the dose ($r = -0.10$, $p > 0.05$), but was 18 per cent lower at hyper- than at normoglycemia (figure 4, $p < 0.01$).

Peripheral Insulin Infusion

PCR_{pe} was much smaller than PCR_{po} following the smallest doses of insulin, whereas the values approached each other after the largest doses (table 2). It was not demonstrably affected by variation of the insulin

dose at normoglycemia ($r = -0.04$, $p > 0.05$), but there was a negative correlation between dose and PCR_{pe} at hyperglycemia ($r = -0.56$, $p < 0.05$). APV_{pe} varied with insulin dose ($r = 0.56$, $p < 0.001$), whereas it did not change during glucose infusion (figure 4).

Relations between Insulin Kinetics and other Metabolic Variables

PCR was tested for possible correlations with k_G (see table 1), pretest blood glucose concentration, pretest endogenous plasma insulin concentration, relative weight, and age. Owing to the findings at hyperglycemia, also APV was tested for possible relations with pretest glucose and insulin concentrations. In order to account for the variation with insulin dose, the latter was included in the partial correlation coefficients given below. After portal insulin infusion, PCR_{po} varied negatively with relative weight (pooling of results at normo- and hyperglycemia: pooled partial $r = -0.42$, $p < 0.01$). The corresponding regression equations (with log transformation of dose and PCR_{po}) predict that an increase of 10 per cent in relative weight gives, on the average, a decrease of about 9 per cent in PCR_{po} . The APV_{po} value obtained at normoglycemia showed a weak negative correlation with fasting blood glucose concentration (partial $r = -0.36$, $p < 0.05$). None of the remaining variables varied significantly with PCR or APV. After peripheral insulin infusion, the pooled partial r between PCR_{pe} and relative weight was -0.19 ($p > 0.05$).

Stepwise multiple regression analysis was performed in order to ascertain to what extent joint relations between the above independent variables influenced the correlations with PCR or APV. It was found that relative weight and fasting blood glucose had stable negative relationships with PCR_{po} and APV_{po} , respectively. For example, the significant negative correlation between PCR_{po} and relative weight was thus not due to joint correlations between relative weight and other variables, such as blood glucose or plasma insulin. It should be noted that in the material as a whole the correlation coefficient between relative weight and fasting plasma insulin was 0.249, which almost reached the 5 per cent level of significance. There was a weak negative correlation between age and PCR_{po} at normoglycemia when the influence of relative weight was eliminated (partial $r = -0.36$, $p < 0.05$).

Relations between PCR and Insulin Sensitivity

The values of blood glucose decrement are summarized in table 2 and their relationships to insulin dose and PCR are given in table 3. Blood glucose decrement, used as an index of insulin sensitivity, showed a good proportionality to insulin dose. The dose was again included in the partial correlations in order to eliminate the effect of different doses on PCR and blood glucose decrement. No significant relationship was found between the blood glucose decrement and PCR. Furthermore, the blood glucose decrement as well as the fasting plasma insulin concentration were smaller in the afternoon than in the morning ($p < 0.05$ and

TABLE 3

Relationships between the blood glucose decrement, an index of insulin sensitivity, and insulin dose or PCR

Route of insulin infusion	Time of day	Glucose infusion	n	Variable	Variable Dose ($\mu\text{U./kg.}$)	PCR [†] ($\text{ml. min.}^{-1} \text{ kg.}^{-1}$)
Portal	a.m.	-	32	Blood	0.85***	-0.04
	a.m.	+	15	glucose	0.73**	-0.37
	p.m.	-	10	decrement,	0.75*	0.38
Peripheral	a.m.	-	41	%	0.72***	-0.07
	a.m.	+	14		0.91***	0.01

PCR, plasma clearance rate.

[†]Partial correlation (the dose was held constant).

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

$p < 0.01$, respectively), whereas PCR, or other kinetic parameter values, did not change. The smaller PCR values at hyperglycemia were not accompanied by any change in the effect of insulin on blood glucose ($p > 0.05$).

The values of FFA decrement (table 2) showed somewhat weaker correlations with dose than did those of the blood glucose decrement, but their partial correlation coefficients with PCR were of similar magnitude and never different from zero (data not shown).

Inguinal Herniorrhaphy

Three days after inguinal herniorrhaphy, PCR_{pe} was of the same size as before operation. The blood glucose decrement was 4.6 ± 3.8 per cent less postoperatively ($p < 0.05$), whereas the kg values did not decrease significantly ($p > 0.05$).

DISCUSSION

This study showed that the non steady-state disappearance of insulin entering the portal vein is modulated by insulin dose and glucose infusion. Thus, both high insulin doses and elevated glucose levels caused a decrease in the clearance rate of insulin. It also showed that PCR_{po} varies negatively with relative weight and age. No such relations were found after peripheral infusion though a combination of increased insulin dose and of glucose infusion was associated with a decrease in PCR_{pe} , too. The liver thus seems to play a major role for the distribution and removal of insulin, at least shortly after changes in portal glucose or insulin. Otherwise, there was a notable lack of correlations between PCR and other factors such as fasting plasma insulin, insulin sensitivity or glucose tolerance.

For obvious reasons the portal studies were limited to patients with malignant disease. But neither the disease nor the preceding catheterization of the portal vein had a demonstrable effect on the variables studied. Inguinal herniorrhaphy, being a substantially longer and more traumatizing procedure, was followed by a slight decrease in insulin sensitivity alone. The kg values were low, but comparable with those found by Palmer and Ensink¹⁶ in normal subjects of similar age. It should be observed that kg is the same whether the IVGTT is performed with or without preceding insulin infusion at the rates used here (unpublished observation).

The utilization of portal and peripheral infusions of insulin made it possible to estimate the importance of the liver for the turnover rate of insulin. PCR_{po} is a combined measure of first pass hepatic uptake and the subsequent removal of insulin in the periphery and insulin recirculating to the liver (see figure 1). The differences between the results of portal and peripheral infusions were thus essentially due to the

amount of insulin removed during the first liver passage after portal infusion. Since PCR_{po} , but not PCR_{pe} , decreased with increasing dose of insulin, it is obvious that this effect was due mainly to a decrease in the first pass fractional hepatic uptake. APV_{pe} increased with the insulin dose, apparently an effect of the reentry of insulin from the extravascular compartment(s).⁹ APV_{po} did not vary with insulin dose, which, again, can only be explained by a diminished first pass fractional hepatic uptake after large insulin doses into the portal vein. During steady hyperglycemia the decrease in PCR_{po} was accompanied by a corresponding decrease in APV_{po} . Since APV_{pe} did not change when the blood glucose level was acutely raised, this shows that the first pass hepatic uptake of insulin was diminished at hyperglycemia. As for PCR_{pe} , the effects of insulin and glucose were demonstrable only after infusion of both. This is not surprising because only one fifth to one fourth of the cardiac output is diverted to the liver, which means that the effect of a reduced hepatic uptake on PCR_{pe} will be comparatively small and masked by uptake in other tissues. A diminished hepatic uptake of insulin in response to an acute increase of glucose has previously been demonstrated.⁷

The above findings infer that when the insulin secretion rate is raised by glucose, the increases in both insulin and glucose will divert a larger fraction of the endogenous insulin from the liver to the peripheral tissues. For insulin concentrations clearly within the physiologic range, as after the two smallest doses (5-10 mU./kg.), the effect of insulin itself on PCR_{po} is at most slight and not proven.⁹ But the marked effect of increased glucose infers that changes in blood glucose cause changes in peripheral insulin concentration that do not reflect changes in pancreatic secretion rate alone. It is presently unknown whether this effect of glucose extends to the diabetic state.

The present study does not reveal whether the diminished hepatic uptake of insulin at hyperglycemia is a direct effect of glucose or a result of changes secondary to the hyperglycemia. The rise in endogenous insulin levels, which might give occupancy or down regulation of liver receptors by insulin, does not appear responsible. Thus, the pretest endogenous plasma insulin (absolute or incremental) showed no relationship to PCR or APV . Studies in vivo^{17,18} and in vitro^{19,20} with insulin concentrations as low as those obtained by the glucose infusions have shown no effect on hepatic uptake within 1-3 hours (portal insulin concentrations averaged 51 μ U./ml. before the infusion of insulin). Down regulation of receptors by insulin itself appears to require more time,^{21,22} and its physiological significance has been questioned.²² Finally, PCR did not vary between morning and afternoon, whereas fasting plasma insulin did.

We have not found any previous report of a relation between the degree of obesity and the plasma insulin disappearance rate in vivo. The findings suggest that the obese patients had a decreased uptake both in peripheral tissues and liver and agree with studies in vitro.¹ The variation of relative body weight accounted for 17-18 per cent of the

variation of PCR_{po} . The negative correlation could not (statistically) be explained by the plasma insulin levels. This may have been due to the fact that none of our patients was remarkably obese and that most of them were within the range of ideal weight. It should be observed that the experimental design might introduce a bias when dose and clearance rate are adjusted to body weight. Owing to the dose-dependence of PCR_{po} , a comparatively small plasma volume per kg. body weight, as in severe obesity, will in itself cause a negative correlation between relative weight and PCR_{po} (in $ml. min.^{-1} kg.^{-1}$). But it is reasonable that the calculation of PCR per kg. body weight was appropriate because plasma and liver volumes are proportional to body weight within the weight range studied.^{23,24} Furthermore, we did not find any correlation between APV (in $ml./kg.$), on one hand, and absolute or relative weight, on the other.

Binding of insulin in vitro is frequently found to be negatively correlated with the fasting insulin concentration and positively with the effect of insulin. There are, however, several situations in which these variables change in opposite directions and/or where these relations are absent.^{1,25-28} Non steady-state PCR was not related to fasting plasma insulin, insulin sensitivity or glucose tolerance. This implies that factors other than the number of insulin receptors are also important for the size of PCR and that variation in PCR is relatively unimportant for the acute effect of insulin on glucose and FFA metabolism. A similar lack of relations was reported by DeFronzo et al.,²⁸ who studied steady-state PCR_{pe} in fasting obese subjects, though it should be noted that they found PCR_{pe} to vary positively with insulin action, and negatively with insulin binding, when starvation lasted for 14 days. Stimmeler et al.⁶ found a correlation between the disappearance and effect of insulin in normals and untreated diabetics, whereas Pearson and Martin⁵ did not. Diabetics have either a normal³⁻⁵ or a decreased^{2,6} removal rate of insulin, and the results do not seem to vary with the fasting plasma insulin concentration.

Possible relationships with non steady-state PCR may be difficult to demonstrate because PCR is influenced by the rate and distribution of blood flow and the rates of exit from and reentry to the plasma volume, in addition to the rate of irreversible binding in different organs. Furthermore, since at least part of the plasma insulin is degraded in extravascular compartment(s), it follows that the larger the extravascular binding the smaller the apparent distribution volume.⁹ Accordingly, the latter might be a more appropriate index of extravascular binding. At any rate, we did not find it to vary with the variables under discussion (data not shown).

We believe that the absence of correlations between PCR and fasting plasma insulin, insulin sensitivity or glucose tolerance is due mainly to the fact that the latter variables are influenced by many metabolic and hormonal factors likely to modulate the release and, perhaps predominantly, the post-receptor fate and effect of insulin.^{1,25-27} In addition, intraindividual variation of the above variables should

be more closely related to variation in PCR if any relationship exists. Thus, lack of a demonstrable relationship does not exclude its existence, but suggests that other factors play significant roles.

It has recently been postulated that the regulation of blood glucose is of secondary importance to the maintenance of optimal (peripheral) plasma insulin levels.²⁹ Our results support this hypothesis. Thus, while glucose is a powerful regulator of plasma insulin, by affecting both the release and the (hepatic) removal of insulin, the relationship between the removal rate and the effect of insulin is less obvious. The preference to divert a larger fraction of secreted insulin to the periphery during hyperglycemia, and a smaller one during normoglycemia, is compatible with the anabolic and catabolic demands in the fed and fasted state.³⁰ It is also compatible with the occurrence of spontaneous hypoglycemia in early diabetes.³¹

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DISAPPEARANCE OF INSULIN IN MAN:
VARIATION WITH THE PLASMA INSULIN LEVEL

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ABSTRACT

Clearance rates of insulin were estimated in unanaesthetized non-diabetic humans. The clearance rate, as well as the pancreatic secretion rate, of endogenous insulin was estimated from steady-state concentrations in portal and arterial blood. The clearance rate of exogenous insulin was determined after brief intraportal infusion (8-43 mU/kg).

In the basal fasting state, inter-individual differences in the height of the endogenous plasma insulin level were markedly related to differences in the rates of both secretion and removal, whereas they were predominantly related to variation in the rate of insulin secretion during glucose infusion. Clearance of exogenous insulin was not related to the pre-test endogenous insulin level but fell progressively with increasing dose, from 35 (8 mU/kg) to 14 (43 mU/kg) $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ at normoglycemia and from 23 (8 mU/kg) to 17 (34 mU/kg) $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ at hyperglycemia. The clearance rate of endogenous insulin was lower in the basal fasting state (11 $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) than during glucose infusion (17 $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$). At normoglycemia, it was lower than the values recorded for exogenous insulin, whereas clearance rates of endogenous and exogenous insulin were similar during glucose infusion.

In conclusion, this study showed that variation in clearance rate is partly responsible for variation in the basal fasting insulin level. It indicated that clearance is lowest in this situation, substantially increased after moderate elevation of plasma insulin and, again, lower after marked plasma insulin elevation. It is, however, possible that the results for endogenous insulin in the basal state reflect poor specificity of the insulin radioimmunoassay at these low concentrations.

Plasma insulin levels show a remarkably large range of variation between individuals, both in health and disease, and it has generally been thought that this reflects variation in the secretion rate of insulin. But recent findings suggest that variation in plasma insulin disappearance rate may play a role (Gavin et al. 1974; Sönksen et al. 1977; Tranberg & Dencker 1978). As for the fate of insulin, uncertainty is due to methodological difficulties (Franckson & Ooms 1973; Navalesi et al. 1977; Sönksen et al. 1977; Tranberg & Dencker 1978), the complexity of the dynamic relationships between insulin and glucose (Sherwin et al. 1974; Hagander et al., in press), species differences (Hiebert et al. 1972; Brockman & Bergman 1975), and the anatomic position of the pancreas.

In the present study, the release and removal of endogenous insulin in the steady-state were determined by means of portal and arterial sampling. The plasma clearance rate after brief intraportal infusion of insulin was also determined. The purposes were a) to estimate the rates of insulin secretion and insulin clearance in the steady-state, b) to elucidate to what extent these kinetic parameters accounted for the height of the plasma insulin concentration, c) to compare the plasma clearance rate of endogenous insulin in the steady-state with that found for exogenous insulin in the non steady-state.

MATERIAL AND METHODS

Subjects. Forty-five in-patients with a transumbilical portal catheter were studied (Table 1). Most of them were to be, or had been, operated upon because of carcinoma of the colon. The portal catheter had been inserted 2 days before portography for possible liver metastases (Göthlin et al. 1975), and 3 days before the experimental studies. Only patients in whom portography, liver scintigraphy and subsequent laparotomy had not demonstrated liver metastases, or porto-systemic shunting, were included.

The day after portal catheterization the patients resumed their pre-operative routine, moved about freely in the ward and consumed an ordinary hospital diet. All had normal fasting blood glucose concentrations. None had previous endocrine disease and none was taking drugs known to affect carbohydrate or insulin metabolism. The results of standard liver and kidney laboratory tests were normal in all. No major operation was performed within the last 6 months before the studies.

The studies were performed in accordance with the principles of the Declaration of Helsinki. The patient's informed consent was verbal.

Experimental protocol. The experimental procedures have previously been given in detail (Tranberg & Dencker 1978). The

Table 1.
Clinical characteristics.

No. of patients	Sex		Age (years)	Relative weight 1)	Fasting arterial blood glucose 2) (mmol/l)	Diagnosis carcinoma ³⁾ miscellaneous ⁴⁾
	M	F				
45	27	18	61.6 ⁺ -12.2	1.07 ⁺ -0.12	4.22 ⁺ -0.60	38 (4) 3
			16-82	0.86-1.41	3.17-5.72	

First line gives means ⁺ SD, second line gives the ranges.

1) Tables of Metropolitan Life Insurance Company.

2) Corresponding values for portal blood: 4.18⁺-0.58 (3.17-5.67). Fasting blood glucose concentration was the same in the afternoon as in the morning.

3) Carcinoma of the colon or, shown within parentheses, of the urinary tract.

4) Crohn's disease 2, hydronephrosis 1.

portal catheter was kept patent by a slow continuous infusion of heparinized saline (5000 IU of heparin each day). During portography the tip (with 2 side-holes within 1.5 cm of the curved end) of the catheter (Medioplast, Denmark, I.D./O.D. = 1.4/2.2 mm) was secured in the main portal vein. After an overnight fast the heparinized solution was replaced by saline. One hour before the start of the study, a radial artery was cannulated for blood sampling.

The sampling schedule is seen in Fig. 1. All studies began with 3 paired samples of portal and arterial blood at 5 min intervals. In 12 patients glucose was then infused intraportally at a rate of 80 or 200 $\text{mg}\cdot\text{h}^{-1}\cdot\text{kg}^{-1}$. Designating completion of sampling in the basal state as time 0, portal and arterial blood were sampled at 10 min intervals between 70-90 and 90-120 min, respectively, when steady levels of glucose and insulin had been obtained (Tranberg & Dencker 1978). Portal blood was sampled 10 s after the arterial. After sampling during steady-state normo- or hyperglycemia, a 2.5 min intraportal infusion of monocomponent porcine insulin (Actrapid®) was given, and followed by frequent sampling of arterial blood.

The 80 and 200 $\text{mg}\cdot\text{h}^{-1}\cdot\text{kg}^{-1}$ glucose infusions raised arterial blood glucose to respectively 5.54 ± 0.41 and 6.63 ± 0.96 mmol/l. During glucose infusion portal blood glucose was about 0.4 and 1 mmol/l higher than the respective peripheral value.

In 14 patients fasting was prolonged to 2 p.m. before the studies were started. Otherwise the studies, including all at hyperglycemia, were started in the morning. The number of experiments in each study group is shown in Tables 2-3.

Analytical methods. Sampling tubes contained EDTA. Blood glucose was determined with a glucose oxidase method (Hjelm & de Verdier 1963). Plasma was separated rapidly and stored at -20°C until triplicate determination of total immunoreactive insulin (Heding 1966). Details of our insulin assay have been presented elsewhere (Tranberg & Dencker 1978).

Calculations. The following symbols are used. PCR_{ss} ($\text{ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$) is the plasma clearance rate of intraportally released insulin in the steady-state. PCR_i ($\text{ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$) is the plasma clearance rate in the non steady-state after a short intraportal insulin infusion of dose D ($\mu\text{U}/\text{kg}$). It should be noted that PCR includes the first pass hepatic clearance rate after intraportal secretion or infusion of insulin. The portal and arterial concentrations of plasma insulin are denoted by I_{po} and I_{a} ($\mu\text{U}/\text{ml}$), respectively, and portal plasma flow by PPF ($\text{ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$). The pancreatic secretion rate into the plasma pool in the steady-state is denoted SR_{ss} ($\mu\text{U}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$).

The over-all PCR_i was defined as (Tranberg & Dencker 1978)

$$PCR_i = D / \int_0^{\infty} (I_a(t) - I_a^{ss}) dt \quad (1)$$

where I_a^{ss} denotes the pre-test steady-state concentration. PCR_i was thus calculated on plasma insulin levels above the pre-test baseline.

Also, by definition:

$$SR_{ss} = PCR_{ss} \cdot I_a^{ss} \quad (2)$$

If it is assumed that there is no net exchange of insulin across the extrahepatic and extrapancreatic splanchnic bed, the following relationship holds:

$$SR_{ss} = PPF (I_{po}^{ss} - I_a^{ss}) \quad (3)$$

It follows from equations 2 and 3 that

$$PCR_{ss} = PPF (I_{po}^{ss} / I_a^{ss} - 1) \quad (4)$$

If the clearance is linear and stationary, PCR_i should equal PCR_{ss} . If it is not, it is difficult to relate the two measurements because PCR_i would correspond to some average of different PCR 's. At steady hyperglycemia it was also of interest to calculate the incremental PCR_{ss} :

$$\Delta PCR_{ss} = PPF \cdot \Delta (I_{po}^{ss} - I_a^{ss}) \quad (5)$$

Statistical methods. Standard statistical procedures were used (Siegel 1956; Snedecor & Cochran 1967). The distributions of fasting I_{ss} , I_a^{ss} , I_{po}^{ss}/I_a^{ss} , and $(I_{po} - I_a)^{ss}$ values were skewed to the right and log transformation created distributions that looked "normal". (Owing to the small number of determinations, the distributions of values at hyperglycemia could not be settled.)

RESULTS

A typical set of data is shown in Fig. 1. Table 2 summarizes the steady-state portal and arterial insulin values. I_{po}^{ss} , I_a^{ss} and $(I_{po} - I_a)^{ss}$ were smaller in the afternoon, whereas the I_{po}^{ss}/I_a^{ss} ratios did not change. The latter were larger at hyper- than at normoglycemia.

As found previously (Tranberg & Dencker 1978), PCR_i values decreased with the dose (Table 3). They did not vary during the day, and were not significantly smaller at hyper- than at normoglycemia (analysis of co-variance, $P > 0.05$).

The coefficient of variation of the mean of I_a^{ss} values was

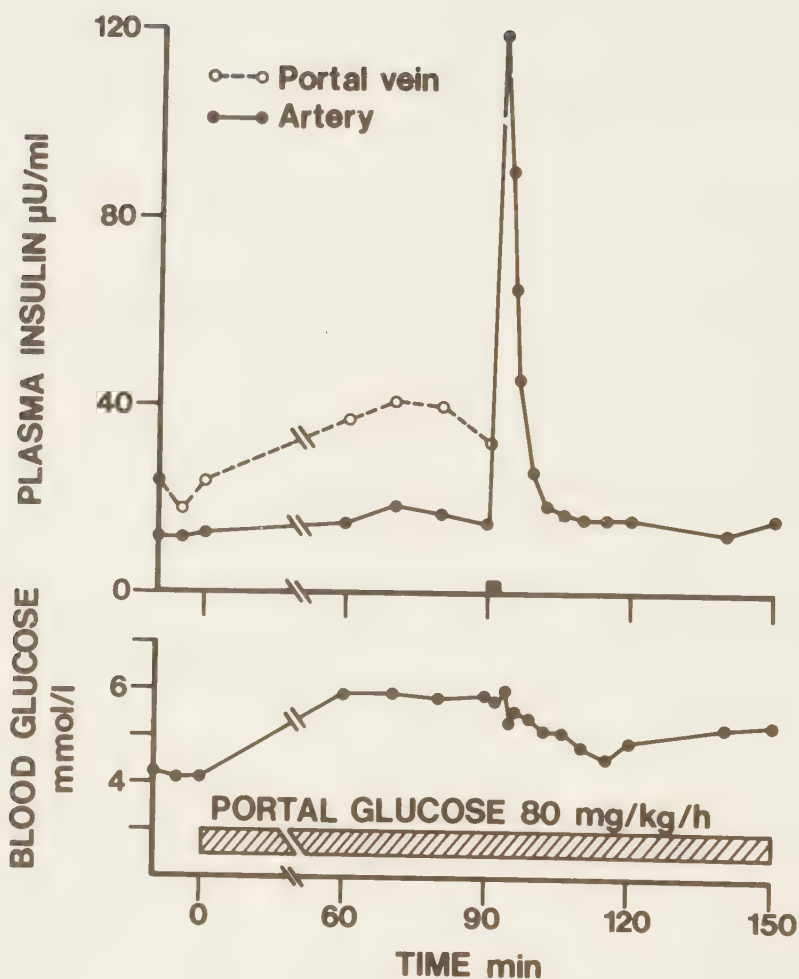


Fig. 1

Time-course of plasma insulin and blood glucose concentrations in one experiment. Glucose was infused into the portal vein from 0 to 150 min. An intraportal infusion of insulin (8.2 mU/kg) was given from 90 to 92.5 min. Filled bar denotes the insulin infusion.

Table 2.

Portal and arterial plasma insulin concentrations, portal-arterial insulin differences and portal-arterial insulin ratios.

Time of day	No. of determinations	I_{po}^{ss} (μ U/ml)	I_a^{ss} (μ U/ml)	$(I_{po} - I_a)^{ss}$ (μ U/ml)	I_{po}^{ss} / I_a^{ss} ratio
Fasting	38 ¹⁾	25.5 (14.3-35.0)	12.0 (6.0-16.0)	12.5 (4.9-21.2)	2.08 (1.58-2.81)
	14	9.0 (5.7-13.0) ***	4.2 (2.0-8.3) ***	5.0 (1.7-10.0) **	2.00 (1.50-3.50)
Glucose ₄₁ 80 mg·h ⁻¹ ·kg ⁻¹	5	31.5 (17.3-51.9)	12.7 (5.5-17.8)	19.3 (11.8-34.1)	2.71 (2.27-3.42) ³⁾
Glucose ₂₀₀ 200 mg·h ⁻¹ ·kg ⁻¹	7	61.3 (46.8-127.6)	25.0 (15.3-47.0)	42.4 (26.9-84.8)	2.71 (2.04-5.05) ³⁾

Values are medians and 20th and 80th percentiles. I_{po} , portal plasma insulin concentration; I_a , arterial plasma insulin concentration.

1) Seven patients were examined twice on two consecutive days.

2) Mann-Whitney test for difference between a.m. and p.m. values: ** $P < 0.01$, *** $P < 0.001$.

3) When pooled, these values are larger than at normoglycemia (Mann-Whitney test, $P < 0.05$).

Table 3.

Plasma clearance rate (PCR_i) after brief, intraportal infusion of insulin.

	Dose (mU/kg)	No. of determinations	PCR_i ($ml \cdot min^{-1} \cdot kg^{-1}$)
Fasting ¹⁾	8.2	4	35.1 ± 11.7 (35.4)
	17.5	3	25.0 ± 7.3 (21.6)
	25.2	7	17.1 ± 5.2 (16.6)
	33.4	2	15.2 ± 5.3
	43.3	4	14.2 ± 3.2 (14.5)
Glucose, 80 and 200 $mg \cdot h^{-1} \cdot kg^{-1}$ ²⁾	8.3	5	23.0 ± 10.9 (19.2)
	16.7	3	18.8 ± 5.0 (17.0)
	33.9	1	16.8

Values are means $\pm$ SD and, within parentheses, medians.

1) Results obtained in the morning and afternoon are pooled because they were similar.

2) The results were similar for the two dose levels of glucose infusion.

15 % at normoglycemia, and 8 % at steady hyperglycemia. For I_{po}^{SS} the corresponding values were respectively 17 and 10 %.

The SR_{SS} and PCR_{SS} values given in Table 4 have been calculated under the assumption that PPF is $10 ml \cdot min^{-1} \cdot kg^{-1}$ (Greenway & Stark 1971; Hiebert et al. 1972). It is known that PPF does not differ much from the basal value when glucose is infused continuously at the rates used here (Felig & Wahren 1971; Kaden et al. 1973; Camu 1975). PCR_{SS} was about $10 ml \cdot min^{-1} \cdot kg^{-1}$ at normoglycemia and about $17 ml \cdot min^{-1} \cdot kg^{-1}$ at hyperglycemia. It is noteworthy that PCR_{SS} and PCR_i did not change from morning to afternoon, whereas SR_{SS} decreased.

Table 4.

Rates of pancreatic secretion and plasma clearance of endogenous insulin.

	Time of day	SR_{ss} ($mU \cdot min^{-1} \cdot kg^{-1}$)	PCR_{ss} ($ml \cdot min^{-1} \cdot kg^{-1}$)
Fasting	a.m.	0.125 (0.049-0.212)	10.8 (5.8-18.1)
	p.m.	0.050 (0.017-0.100)	10.0 (5.0-25.0)
Glucose, 80 $mg \cdot h^{-1} \cdot kg^{-1}$	a.m.	0.193 (0.118-0.341)	17.1 (12.7-24.2)
Glucose, 200 $mg \cdot h^{-1} \cdot kg^{-1}$	a.m.	0.424 (0.269-0.848)	17.1 (10.4-40.5)

Values are medians and 20th and 80th percentiles. SR_{ss} , pancreatic secretion rate in the steady-state; PCR_{ss} , plasma clearance rate in the steady-state. Values are based on the assumption that portal plasma flow (PPF) is $10 \text{ ml} \cdot min^{-1} \cdot kg^{-1}$.

Relationship between PCR_{ss} and PCR_i . Tables 3 and 4 show a considerable difference between PCR_{ss} and PCR_i . Though PCR_{ss} differed significantly between individuals, there was no correlation between PCR_{ss} and PCR_i in a given patient, neither at normoglycemia ($n = 20$, partial $r = 0.32$, $P > 0.05$ ¹⁾) nor at hyperglycemia ($n = 9$, partial $r = -0.48$, $P > 0.05$). Not even in the 9 patients receiving the smallest dose (8 mU/kg), which produced insulin concentrations well within the physiologic range (Tranberg & Dencker 1978), was there any correlation between PCR_i and PCR_{ss} . In a previous study, 16 patients given "physiologic" doses had a mean PCR_i value of about $30 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ at normoglycemia (Tranberg & Dencker 1978). This value is larger than the PCR_{ss} value obtained at normoglycemia (Mann-Whitney test, $P < 0.001$). In contrast, at hyperglycemia the 8 mU/kg dose gave PCR_i values that did not differ significantly from the corresponding PCR_{ss} values.

Relationships with endogenous insulin concentration and relative weight. The relationships between I_a^{ss} and SR_{ss} , or PCR_{ss} , are given in Table 5. In the basal state the resultant plasma insulin concentration (I_a^{ss}) was related to the clearance rate (PCR_{ss}) as well as to the pancreatic secretion rate (SR_{ss}). The association between I_a^{ss} and SR_{ss} was stronger ($r = 0.56$, $P < 0.001$) when morning and afternoon data were pooled, whereas that between I_a^{ss} and PCR_{ss} was unchanged. During glucose infusion, I_a^{ss} varied significantly only with SR_{ss} . It is noteworthy that fasting and glucose-stimulated I_a^{ss} levels showed similar relationships with the PCR_{ss} value obtained at hyperglycemia ($r = -0.45$ and $r = -0.40$, respectively, $P > 0.05$ in both cases). PCR_i showed no association with I_a^{ss} (or I_{ss}), neither in the basal state (partial $r = -0.24$, $P > 0.05$) nor at steady hyperglycemia (partial $r = 0.00$, $P > 0.05$).

Relative weight was associated with fasting I_a^{ss} (pooling of morning and afternoon results: pooled $r = 0.32$, $P < 0.05$) and fasting SR_{ss} (pooled $r = 0.40$, $P < 0.01$), but not with PCR_{ss} (pooling of results at normo- and hyperglycemia: pooled $r = 0.01$, $P > 0.05$). Again, there was a discrepancy between PCR_{ss} and PCR_i , the latter being negatively correlated with relative weight (pooled partial $r = -0.55$, $P < 0.01$).

DISCUSSION

This study showed that the fasting plasma insulin level is related not only to the insulin secretion rate but also to the plasma clearance rate. This implies that the clearance rate has a significant influence on the height of the fasting plasma insulin concentration. It was also shown that endogenous insulin disappeared more slowly in the basal unper-

¹⁾ Since PCR_i varied with the dose (see Table 3), partial correlation was necessary.

Table 5.

Correlation coefficients (r:s) between the arterial plasma insulin concentration (I_a^{ss}) and the pancreatic secretion rate (SR_{ss}), or the plasma clearance rate (PCR_{ss}), in the steady-state.

		Variable	
		SR_{ss}	PCR_{ss}
Fasting 1)	I_a^{ss}	0.43**	-0.45**
Glucose infusion	I_a^{ss}	0.63*	-0.40

SR_{ss} calculated by equation 3, PCR_{ss} by equation 4.

1) The correlation coefficients for the results obtained in the morning and afternoon were pooled because they could not be proved different.

* $P < 0.05$, ** $P < 0.01$.

turbed state than during glucose infusion. In addition, endogenous insulin disappeared more slowly than exogenous insulin in the basal state, whereas the rate of disappearance of endogenous insulin approached that of exogenous insulin during glucose infusion.

Equations 1-4 are simple and involve a number of assumptions which might affect the results in absolute terms. As for endogenous insulin, errors may be due to inaccuracy of portal sampling and ignorance of factors such as interference of proinsulin in the determination of total insulin immunoreactivity, exchange of insulin across the extrahepatic splanchnic bed, and variation in portal blood flow. The assumption that portal venous blood is well-mixed is supported by previous studies (Hiebert et al. 1972; Strandell et al. 1973;

Fischer et al. 1975). Since no lymphatics have been found in the islets of Langerhans (Rusznayk et al. 1960), all endogenous insulin should pass the portal vein before reaching the systemic circulation. Proinsulin reacts with our anti-serum and its clearance rate is only about one fifth that of insulin (Sönksen et al. 1973). Yet the influence on the PCR_{ss} values (equation 4) is presumably only slight because proinsulin is a small fraction of the total immunoreactive content in portal and peripheral blood, regardless of the rate of insulin secretion (Horwitz et al. 1975). When the plasma insulin concentration is markedly increased by exogenous insulin there appears to be an uptake of insulin in the extra-hepatic splanchnic bed (Ooms et al. 1968; Harding et al. 1975). However, in the basal state, or at insulin concentrations below 50 $\mu U/ml$, the mesenteric insulin extraction is either absent or so small as to be undeterminable (Fischer et al. 1974; Brockman & Bergman 1975; Harding et al. 1975). Different techniques give different values for portal and/or hepatic blood flow and the PPF value chosen by us is a relatively high one (Greenway & Stark 1971; Hiebert et al. 1972). Since the hepatic blood flow is unchanged or at most slightly decreased during continuous glucose infusion (Felig & Wahren 1971; Kaden et al. 1973, Camu 1975), it would not influence the conclusions from comparison between normo- and hyperglycemia. Summing up, the above considerations indicate that $(I_{po} - I_a)^{ss}$ and $(I_{po}^{ss}/I_a^{ss} - 1)$ were appropriate as indices of respectively the rates of pancreatic secretion and plasma clearance in the steady-state.

As for exogenous insulin, it is well-known that labelled insulin returns to the plasma from extravascular pools (Navalesi et al. 1978). On the other hand, when unlabelled insulin is injected more than one component of the plasma decay curve is hard or impossible to determine, especially after small ("physiologic") doses of insulin (Tranberg & Dencker 1978). It is thus possible that failure to detect re-entry to the plasma pool gives fictitiously large PCR_i values for unlabelled insulin. But, as discussed elsewhere (Tranberg & Dencker 1978), the magnitude of this bias appears negligible, indicating that the PCR_i values predominantly reflect irreversible loss from plasma. It is likely, but not proved, that porcine insulin behaves like human insulin in man. The disappearance from plasma (Sönksen et al. 1973; Sherwin et al. 1974) as well as the ability to cross vascular membranes (Rasio et al. 1967) are the same for porcine and human insulin.

Glucose infusion was associated with a PCR_{ss} value that was similar to that of PCR_i in this situation and larger than the PCR_{ss} value in the basal fasting state. In a study in dogs, PCR_{ss} was as high as 28 $ml \cdot min^{-1} \cdot kg^{-1}$ following continuous intraportal infusion of unlabelled insulin (Harding et al.

1975), whereas it can be calculated that it was $15-16 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ in the basal unperturbed state (equation 4). It thus appears that the clearance rate of plasma insulin is not the same for basal and elevated insulin levels and that the elimination of immunoreactive insulin is nonlinear in the following way. It is lowest in the basal state, increases considerably after moderate elevation of plasma insulin and decreases again after marked elevation of insulin.

The observation that the clearance rate of immunoreactive insulin is lower in the basal state than otherwise can be given only hypothetical explanations, one being related to the specificity of the immunoassay and others to the kinetics of insulin. High-molecular-weight complexes of insulin (Antonides 1976; Smith et al. 1978), modified insulins (Steiner et al. 1968) and unspecified substances in plasma (Sankaran & Harrington 1976) may interfere with the insulin immunoassay. Such interference is most marked at concentrations close to the detection limit of the assay, but the quantitative effect of any interference is hard to evaluate. The radioreceptor assay gives markedly lower insulin values than the immunoassay in fasting blood (Ozaki & Kalant 1977). Unfortunately, the interpretation is hampered by the comparatively low sensitivity of the radioreceptor assay at low insulin concentrations. Another explanation is that of reversible binding of insulin. At low insulin concentrations there may be a release from insulin depots that is more or less hindered by increments in the plasma or tissue concentration of insulin. Uptake of insulin across the dog hindlimb or the human forearm appears to be reversible (Rasio 1969; Sönksen et al. 1971; Rasio et al. 1972). But only a small amount of insulin can be displaced into venous blood (Sönksen et al. 1971), and displacement into lymph has not been detected (Rasio 1969). When fasting was prolonged from morning to afternoon the PCR_{SS} value was unchanged though fasting plasma insulin decreased, which also speaks against any significant influence of reversible insulin binding. Finally, it should be mentioned that Izzo et al. (1967) argued that parts of the insulin removal system in the body do not react below a certain critical concentration. The suggestions that nonspecific binding to antibodies, or a very slow re-entry from insulin depots, may play a role are both supported by the following observation: at steady hyperglycemia the PCR_{SS} value calculated on the incremental insulin concentrations (equation 5) was almost identical with the PCR_i value.

Fasting plasma insulin concentrations varied just as closely with insulin clearance as with insulin release, suggesting that variation in clearance is as important as variation in release in determining the fasting plasma insulin concentration. However, the observed diurnal variation of plasma insulin as well as the acute change in response to glucose

appeared to be caused mainly by variation in the secretion rate of insulin.

The above results support the concept of insulin receptor regulation by insulin itself (Gavin et al. 1974), and indicate that this autoregulation plays a significant role in normals. In the afternoon the basal PCR_{SS} value was unchanged, whereas the pancreatic secretion rate and the plasma insulin level were lower. Thus, a change in the number and/or affinity of insulin receptors seems to require more than a few hours to develop. Similar results have been obtained in vitro (Gavin et al. 1974). In addition, the absence of any correlation between pre-test endogenous insulin levels and PCR_i as well as between PCR_{SS} and PCR_i suggests that the disappearance of acutely elevated insulin concentrations depends predominantly on factor(s) other than the insulin receptor.

Relative body weight showed a strong negative correlation with PCR_i , but no correlation with PCR_{SS} . Neither the absence of correlation between relative weight and PCR_{SS} , nor the positive correlation between relative weight and SR_{SS} , are likely to have been due to the assumption of a constant PPF value (in $ml \cdot min^{-1} \cdot kg^{-1}$) because hepatic blood flow increases in obesity, roughly in proportion to body weight in persons that are not markedly obese (Alexander 1963). It may appear surprising that PCR_{SS} was negatively correlated with the height of the fasting insulin level but not with relative weight. The result is, however, consistent with the recent findings of Baxter et al. (1978) that hyperinsulinemia and obesity are not always associated and that variation in receptor number is related to insulin concentration but unrelated to the degree of obesity, absolute body weight or adipose cell size. It thus appears that the increased fasting plasma insulin levels in the obese patients were due mainly to an increased pancreatic secretion rate. On the other hand, our results suggest that a somewhat reduced clearance rate contributes to the comparatively high insulin concentrations seen in obese patients acutely after pancreatic stimulation.

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